Original Article

Significance of ACTN3 gene and its relation with physiological and physical variables as a genetic marker for soccer players' selection at high altitudes

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Abstract:

A huge number of studies were conducted to realize if the ACTN3 gene polymorphism distribution could be a genetic marker for soccer players' selection, some studies have confirmed and some have denied using ACTN3 for players selection at least alone. Hence the researcher in this study tried to investigate the existence of a relation between the ACTN3 gene polymorphism and high altitude in Albaha province (2270 m) in the kingdom of Saudi Arabia, also if there is a relation between this gene and some physiological and physical variables which could be relayed on as a base for players selection. 68 healthy male Saudi soccer players aged between 18.86±2.35 years, playing at the first division of the Saudi League for youth were enrolled in this study. Eleven physiological and physical variables were evaluated including concentration and size of ACTN3 gene, Speed 30 m, Cardio respiratory endurance, Vo₂ max, Lactic acid concentration after effort and the Vital Capacity. The results indicate the existence of a correlation between the ACTN3 gene polymorphism and some physical and physiological variables for the RR genotype followed by the RX genotype. While the only correlation the ACTN3 XX genotype has showed was with the aerobic ability. Finally the researchers confirmed on the importance of the ACTN3 gene polymorphism as a genetic marker for soccer players selection in high altitude in addition to performance skills and recommend that training programs should be updated considering quantifying and distributing the training loads to keep up with the high altitudes.

KeyWords: -ACTN3, High altitude, genetic marker, Vo₂max, lactic acid

Introduction

Athletes' performance is affected by both the genetic makeup of the individual and the environmental factors. Current researches and interests in sports genetics focus on genetic variants that may make a significant contribution to the athlete performance (Lippiet al., 2009; Guth & Roth, 2013). ACTN3 gene is one of the most popular genes. It encodes a member of the alpha-actin binding protein gene family, the alpha-actinin-3 molecular protein. It is only found in fast twitch muscle fibers, functioning as a structural component of sarcomeric Z line affecting muscle fiber composition (Pitsiladis et al., 2013). ACTN3 can be found in the human body as one of three genotypes XX, RR, and RX. RR and RX have been linked to activities in which strength and speed are predominant, while XX has been linked to activities with a predominance of aerobic endurance, in other words people with the XX genotype can't produce ACTN3 protein, and may produce more ACTN2 protein which increases their endurance capabilities (Yanget al., 2003; Ben-Zaken et al., 2015).

Soccer is the most popular game all over the world played by men, women, children and adults with different levels of expertise. Soccer is characterized as high intensity actions, intermittent non-continuous exercise because of the long distances the player is required to cover in 90 minutes. In higher levels of competition there are a great number of sprints, jumps, one-to-one disputes, kicking the ball, tackles and headings. Therefore the performance of soccer players is directly influenced by the predominance of aerobic and anaerobic capacities including power, speed, strength and endurance (Stølen et al., 2005).

Different previous studies, have investigated the relation between ACTN3 with its three genotypes and soccer in different aspects as the players performance in matches, training loads adjustment, severity of injuries, exercise adaptation, and exercise recovery. As presented in these studies, researchers differed in their opinions about considering the ACTN3 as a genetic marker for soccer players selection, some agreed as in (Massidda et al., 2012) discussed the relevance between ACTN3, ACE and BDKRB2 genes polymorphism distributions and explosive leg-muscle strength in 42 top-level Italian soccer players and 106 sedentary healthy nonathletic. Results indicated that the interaction of ACTN3 and ACE polymorphism distributions may have a role in differentiating elite soccer performance quantitative traits.

In (Ulucanet al., 2015) investigated the distribution of ACE and ACTN3 polymorphism in 25 professional male soccer players of Turkish pedigree. Polymerase chain reaction (PCR) was used for the characterization of ACTN3 while single PCR for ACE. Results indicated that ACE and ACTN3 genotypes should be used as genetic markers for selecting successful soccer players. Also (Galeandro et al., 2017) investigated the association between the ACTN3 and ACE, on 43 soccer players and 128 healthy nonathletic. The results indicated that the soccer players showed inclination to the expansion of ACTN3 RR and ACE DD genotypes both independently and in co-occurrence. Based on these results the authors clarified that ACTN3 and ACE genotypes may be a channel to predict soccer players' talent and could be a base to customize types and training times for soccer players.

Wei in (Wei, 2018) inspected the relation between ACE ID and ACTN3 R577X polymorphism distribution with the performance of 92 Chinese elite female soccer athletes as the experimental group and 200 Chinese females as the control group. Results indicated that the combination of ACE II/ID and ACTN3 RR/XR should be a synergetic determinant of the athletic performance of females in soccer.

In (Jeremic et al., 2019) the researchers investigated the underwent acceleration, speed, strength, anaerobic power and aerobic endurance tests of 27 young female soccer players aged 16-18 with different ACE/ACTN3 gene profiles. Results indicated that it is the phenotype-genotype interaction that makes a successful female soccer player. (Pimenta et al., 2013) compared the polymorphism distribution of the ACTN3 with Speed, jump, and endurance test results performed by 200 professional Brazilian players of first division. Results indicated that players having RR genotype are the fastest in short distances while players having XX presented higher rates of Vo_2 max, than players having RR and RX.

While (Koku et al., 2019) denied that ACTN3 could be a genetic marker for soccer players selection through their study which, investigated the distribution of ACTN3 polymorphism distribution between 100 soccer players and 101 sedentary individuals. Results indicated that there was no difference for the ACTN3 distribution between the soccer players and the sedentary individuals and it shouldn't be considered as a genetic marker for athletic performance in soccer. Although there was statistically significant difference in the shorter countermovement jump with arm swing scores between the players having RR and RX.

The effects of high altitude on humans are considerable with the high altitude, the pressure of oxygen in air changes, causing the oxygen molecules to be apart as there is less pressure to push them together. As the human body reaches around 2,100 m above sea level; the saturation of oxyhemoglobin begins to decrease rapidly (Peacock, 1998; Young, & Reeves, 2002). However, the human body has both short-term and long-term adaptations to altitude that allow it to partially compensate for the lack of oxygen (Keet. al., 2017).

A few studies have discussed the effect of long-term adaptations to altitude on healthy humans especially athletes indicating that the athletes bodies have adapted to compensate for hypoxic by increasing minute ventilation, cardiac output, Red Blood Cell (RBC) count, Hemoglobin (Hb) concentration, Maximal Oxygen Consumption (Vo_2 max), and transiently decreasing cognitive performance of the Central nervous system (Mairbäurl, 2013; Parket. al., 2016).

(Bohner et al., 2015) investigated the effect of high altitude on soccer game activity profiles of NCAA Division I female soccer players through comparing between two matches, the first was played at sea level while the other was played at a moderate altitude (1839 m). The results indicated that the teams residing at sea level and competing at a moderate altitude may have a reduced ability in distance covered and a high intensity run rate. While (Williams, 2011) discussed the impact of altitude change, on professional athletes through analyzing the South American international match outcomes between the same teams but played at different altitudes within the same country. Also (Aughey et al., 2013; Buchheit et al., 2013; Gore et al., 2013; Sargent et al., 2013; Wachsmuth et al., 2013) published five research papers introducing the results of a three years multinational collaboration study investigating the alteration of running performance of the Bolivian U20 and the Australian U17 soccer teams playing three matches at La Paz (3600 m), and two matches at the sea level at Santa Cruz (430 m). Results were described in four different terms including game and training running, blood, sleep patterns and acclimatization; recommending that teams should have sufficient time to stabilize and adapt after arrival at altitude as 12 days is insufficient to reach chronic levels of adaption.

The same recommendations were set by (Nassis, 2013), as he examined the effect of altitude on soccer performance during the 2010 World Cup in South Africa, where matches were played at 0,660, 1200–1400, and 1401–1753 m. Results were described in terms of endurance technical skills and errors made by the goalkeepers that resulted in goals conceded. In (Honda et al., 2017) the researchers have also investigated the 2010 FIFA World Cup in their study but concentrated on customization the pre-acclimatization programs of the Japan's National Team performed at a simulated altitude 2000 m. The obtained results indicated that hypoxic ventilatory response, blood lactate concentrations heart rate and oxygen saturation levels were severely affected in some players, so the authors recommended that the pre-acclimatization programs should be individually as some players may be affected with altitude than others.

Finally (Aswal et al., 2017) compared the peak expiratory flow rate and vital capacity of soccer players at different altitudes. The experiment were performed in two different Indian cities Gwalior (196 m) and

Uttarakhand (1700m) using 20 soccer players 10 from each city. The results indicate that the player of Uttarakhand have better peak expiratory flow rate and vital capacity than the players of Gwalior.

Although of the diversity of topics investigated by these studies, none of these studies have exposed before to the ACTN3 polymorphism distribution in soccer players at high altitude and as the researchers already live at high altitude, in Albaha province (2270 m) and daily witness and suffer from the side effects of hypoxia. Also as the researchers work in the field of sport as professional soccer coaches they become interested in investigating the relation between the ACTN3 polymorphism distribution and some physiological and physical variables of soccer players in high altitude.

The remainder of this paper is organized as follows: Section 2 outlines our methodology including sample subjects description. Section 3 presents the obtained results and section 4 analyses these results. Section 5 concludes this paper and outlines possible future work.

Material & methods

Research type and selection of subjects

This study is a descriptive study, performed on 68 healthy male soccer players aged between 18.86 ± 2.35 years, body weight between 71.43 ± 2.63 and height between 174.6 ± 3.22 . The subjects of the sample are Saudi citizens playing at first division of the Saudi League for youth. Their descendants are from Albaha province to assure the homogeneity of the genetic effect as clarified in Table 1, where the skewness coefficient of the sample is from -0.14 to +1.46, ranging between (± 3), which confirms the homogeneity of the research sample.

TABLE 1. The research sample homogeneity

Dependent Variable	N	Mean $\pm$ SD	Skewness
Concentration of ACTN3 Gene	61	590.9 ± 184.2	-.14
Size of ACTN3 Gene	61	$.49 \pm 0.08$	.29
Pulse comfort	68	78.4 ± 5.3	-.61
Aerobic capacity	68	50.2 ± 4.6	-.53
Anaerobic capacity	68	139.3 ± 5.6	.23
Vital Capacity	68	4.2 ± 0.35	.64
Lactic acid	68	7.6 ± 1.5	-.43
Speed 30 m	68	3.6 ± 0.06	1.46
Vertical jump	68	51.7 ± 3.01	-.59
Speed Endurance	68	4.5 ± 0.04	-.41
Cardio respiratory endurance	68	2327.3 ± 22.8	1.19

Measurements

ACTN3 gene polymorphism distribution was detected using PCR at a private laboratory at Albaha province considering the concentration and size of it. The rest of the evaluated variables were split into two types, physical variables and physiological variables. The physical variables were evaluated using Speed test (Speed 30 m), Power test (Vertical jump), Speed Endurance test (5x30 msprint) and Endurance test (Cardio respiratory endurance). While the physiological variables were evaluated using Pulse test (Pulse comfort), Aerobic Capacity test (Vo_2 max), Anaerobic Capacity test (Lewis formula measures), Lactic Acid test (Lactic acid concentration after effort) and Vital Capacity test using the Spirometry.

Albaha province

Albaha Province is situated in the southwest in the Saraa mountain range in KSA, the surface of the earth ranges between 1800 and 2500 m above sea level and constitutes fog in the winter. It has a good climate with temperature from 12 to 23 degrees. As a result of the high altitude of Albaha most of the residents suffer from simple symptoms as Headache, dizziness, fatigue, and sleep disorders to severe diseases as respiratory diseases, heart disease, Cerebral edema, uncontrolled high blood pressure, chronic obstructive pulmonary, sleep apnea, and stroke (Albaha Chamber, 2019; Mount Shada in Al-Baha, 2019).

Statistical analysis

The variables under consideration were statistically analyzed using mean, standard deviation (SD), skewness and Least Significant Difference (LSD) in addition to the Pearson Correlations coefficient and ANOVA test using IBM SPSS Statistics V25 (IBM SPSS Statistics version 25, 2019) where the level of statistical significance was set as $p < 0.05$.

Results

As mentioned in the previous section 11 variables were evaluated for 68 healthy male soccer players, the ACTN3 gene polymorphism distribution results indicated that 33 of the players have RR, 28 have RX while the rest 7 have XX. Table 2 clarifies the homogeneity of the research sample in all variables under consideration for the RR, RX and XX genotypes respectively.

TABLE 2. The research sample homogeneity for the RR, RX and XX genotypes

Dependent Variable	ACTN3 RR		ACTN3 RX		ACTN3 XX	
	N	Mean $\pm$ SD	N	Mean $\pm$ SD	N	Mean $\pm$ SD
Size of ACTN3 Gene	33	753.3 $\pm$ 50.7	28	399.6 $\pm$ 47.2	-	-
Concentration of ACTN3 Gene	33	0.53 $\pm$ 0.07	28	0.4 $\pm$ 0.06	-	-
Pulse Rate	33	82.4 $\pm$ 2.6	28	76.3 $\pm$ 2.9	7	68 $\pm$ 1.6
Aerobic capacity	33	47.3 $\pm$ 4.4	28	52.2 $\pm$ 2.4	7	56.1 $\pm$ 2.7
Anaerobic capacity	33	142.6 $\pm$ 5.3	28	137.5 $\pm$ 2.7	7	130.8 $\pm$ 3.7
Vital Capacity	33	4.01 $\pm$ 0.20	28	4.31 $\pm$ 0.32	7	4.8 $\pm$ 0.2
Lactic acid	33	8.6 $\pm$ 0.91	28	7.1 $\pm$ 1.1	7	4.8 $\pm$ 0.5
Speed 30 m	33	3.57 $\pm$ 0.02	28	3.6 $\pm$ 0.04	7	3.7 $\pm$ 0.08
Vertical jump	33	53.54 $\pm$ 2.1	28	51.07 $\pm$ 1.5	7	45.8 $\pm$ 2.1
Speed Endurance	33	4.6 $\pm$ 0.02	28	4.5 $\pm$ 0.03	7	4.6 $\pm$ 0.02
Cardio respiratory endurance	33	2311.5 $\pm$ 8.6	28	2332.5 $\pm$ 10.9	7	2381 $\pm$ 5.09

Table 3 clarifies that there are differences in ACTN3 size and concentration between the RR and RX genotypes for the RR genotype, as the ACTN3 size average for RR genotype is 753.33 and the ACTN3 size average for RX genotype is 399.64 while the ACTN3 concentration average for RR genotype is 0.53 and the ACTN3 concentration average for RX genotype is 0.45.

TABLE 3. The differences indicators between ACTN3 RR and RX genotypes

Dependent Variable	GROUP	N	Mean	Std. Deviation	Mean Difference	t
Concentration of ACTN3 Gene	RR	33	753.33	50.77	353.69	28.01
	RX	28	399.64	47.17		
Size of ACTN3 Gene	RR	33	.53	.08	.08	4.36
	RX	28	.45	.06		

Table 4 clarifies the existence of statistically significant differences between the three genotypes RR, RX and XX in the physiological and physical variables under consideration, where the highest calculated F value is for the physical variable, the cardio respiratory endurance with 164.75 while the least calculated F value is for the physiological variable, the aerobic capacity with 25.08.

TABLE 4. Differences between ACTN3 RR, RX and XX genotypes in the physiological and physical variables

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Dependent Variable		Sum of Squares	df	Mean Square	F
Pulse Rate	Between Groups	1416.26	2	708.13	96.98
	Within Groups	474.61	65	7.31	
	Total	1890.87	67		
Aerobic capacity	Between Groups	626.03	2	313.05	25.08
	Within Groups	811.21	65	12.48	
	Total	1437.24	67		
Anaerobic capacity	Between Groups	943.29	2	471.65	25.64
	Within Groups	1195.59	65	18.39	
	Total	2138.88	67		
Vital Capacity	Between Groups	4.05	2	2.02	29.48
	Within Groups	4.46	65	.07	
	Total	8.51	67		
Lactic acid	Between Groups	99.03	2	49.51	51.03
	Within Groups	63.07	65	.97	
	Total	162.09	67		
Dependent Variable		Sum of Squares	df	Mean Square	F
Speed 30 m	Between Groups	.17	2	.09	43.75
	Within Groups	.13	65	.01	
	Total	.29	67		
Vertical jump	Between Groups	362.34	2	181.17	48.09
	Within Groups	244.89	65	3.77	
	Total	607.24	67		
Speed Endurance	Between Groups	.08	2	.04	55.17
	Within Groups	.05	65	.01	
	Total	.13	67		
Cardio respiratory endurance	Between Groups	29165.27	2	14582.64	164.75
	Within Groups	5753.24	65	88.51	
	Total	34918.52	67		

The results indicate that there are statistically significant differences in anaerobic capacity (anaerobic capacity - speed – speed endurance - power) between the three genotypes of the ACTN3 for the RR and RX genotypes. There are also statistically significant differences in aerobic capacity (pulse comfort-Vo₂max –vital capacity-power) between the three genotypes of the ACTN3 for the XX.

TABLE 5.LSD between ACTN3 RR, RXand XX genotypes in physiological & physical variables

Dependent Variable	Mean $\pm$ SD		(I) GROUP	(J) GROUP	Mean Difference (I-J)
Pulse Rate	82.4 $\pm$ 2.6	LSD	RR	RX	6.09*
				XX	14.45*
	76.3 $\pm$ 2.9		RX	RR	- 6.09*
				XX	8.35*
	68 $\pm$ 1.6		XX	RR	- 14.45*
				RX	- 8.35*
Aerobic capacity	47.3 $\pm$ 4.4	LSD	RR	RX	- 4.85*
				XX	- 8.77*
	52.2 $\pm$ 2.4		RX	RR	4.85*
				XX	- 3.92*
	56.1 $\pm$ 2.7		XX	RR	8.77*
				RX	3.92*
Anaerobic capacity	142.6 $\pm$ 5.3	LSD	RR	RX	5.03*
				XX	11.74*
	137.5 $\pm$ 2.7		RX	RR	- 5.03*
				XX	6.71*
	130.8 $\pm$ 3.7		XX	RR	-11.74*
				RX	-6.71*
Vital Capacity	4.01 $\pm$ 0.20	LSD	RR	RX	-.29*
				XX	-.79*
	4.31 $\pm$ 0.32		RX	RR	.29*
				XX	-.50*
	4.8 $\pm$ 0.2		XX	RR	.79*
				RX	.50357*
Lactic acid	8.6 $\pm$ 0.91	LSD	RR	RX	1.58*
				XX	3.83*
	7.1 $\pm$ 1.1		RX	RR	- 1.58*
				XX	2.25*
	4.8 $\pm$ 0.5		XX	RR	- 3.83*
				RX	- 2.25*
Speed 30 m	3.57 $\pm$.002	LSD	RR	RX	-.045*
				XX	-.169*
	3.6 $\pm$ 0.04		RX	RR	.045*
				XX	-.124*
	3.7 $\pm$ 0.08		XX	RR	.16*
				RX	.12*
Vertical jump	53.54 $\pm$ 2.1	LSD	RR	RX	2.47*
				XX	7.68*
	51.07 $\pm$ 1.5		RX	RR	- 2.47*
				XX	5.21*
	45.8 $\pm$ 2.1		XX	RR	- 7.68*
				RX	- 5.21*
Speed Endurance	4.6 $\pm$.002	LSD	RR	RX	.06*
				XX	-.02
	4.5 $\pm$ 0.03		RX	RR	-.06*
				XX	-.084*
	4.6 $\pm$ 0.02		XX	RR	.09
				RX	.08*
Cardio respiratory endurance	2311.5 $\pm$ 8.6	LSD	RR	RX	-20.98*
				XX	- 69.48*
	2332.5 $\pm$ 10.9		RX	RR	20.98*
				XX	- 48.50*
	2381 $\pm$ 5.09		XX	RR	69.48*
				RX	48.50*

Table 5 clarifies the existence of LSD at (0.05) between the ACTN3 three genotypes RR, RX and XX in (anaerobic capacity - lactic acid –speed 30 m- power) for the RR. also the existence of LSD between the ACTN3 three genotypes RR, RX and XX in speed endurance for the RX. And the existence of LSD between the ACTN3 three genotypes RR, RX and XX in (pulse comfort - aerobic capacity – vital capacity - cardio respiratory endurance) for the XX.

TABLE 6. The relation between the ACTN3 RR genotype and the physiological and physical variables

Dependent Variable	Concentration of ACTN3 Gene	ACTN3 Gene	Pulse Rate	Aerobic capacity	Anaerobic capacity	Vital Capacity	Lactic acid	Speed 30 m	Vertical jump	Speed Endurance	Cardio respiratory endurance
Concentration of ACTN3 Gene	1	-.038	.068	-.337	-.011	-.066	.364*	.224	-.178	.047	-.147
N	33	.832	.707	.055	.951	.715	.037	.210	.323	.795	.415
Size of ACTN3 Gene	-.038	1	.197	.066	-.164	.306	.110	-.236	.085	-.024	.025
N	.832	.832	.272	.715	.361	.084	.543	.186	.638	.893	.892
	33	33	33	33	33	33	33	33	33	33	33
Pulse Rate	.068	.197	1	.147	.381*	-.055	.189	.104	-.336	.172	-.055
N	.707	.272	.33	.414	.029	.760	.293	.563	.056	.338	.762
	33	33	33	33	33	33	33	33	33	33	33
Aerobic capacity	-.337	.066	.147	1	.101	-.034	.013	-.026	.158	.082	.329
N	.055	.715	.414	.33	.577	.850	.945	.887	.380	.649	.061
	33	33	33	33	33	33	33	33	33	33	33
Anaerobic capacity	-.011	-.164	.381*	.101	1	-.083	.007	.174	-.211	.081	.118
N	.951	.361	.029	.577	.33	.646	.970	.334	.237	.654	.513
	33	33	33	33	33	33	33	33	33	33	33
Vital Capacity	-.066	.306	-.055	-.034	-.083	1	.208	.077	.328	.292	.244
N	.715	.084	.760	.850	.646	.33	.246	.668	.062	.099	.171
	33	33	33	33	33	33	33	33	33	33	33
Lactic acid	.364*	.110	.189	.013	.007	.208	1	.327	-.150	.242	-.220
N	.037	.543	.293	.945	.970	.246	.33	.063	.405	.174	.219
	33	33	33	33	33	33	33	33	33	33	33
Speed 30 m	.224	-.236	.104	-.026	.174	.077	.327	1	-.157	.210	-.238
N	.210	.186	.563	.887	.334	.668	.063	.33	.383	.241	.183
	33	33	33	33	33	33	33	33	33	33	33
Vertical jump	-.178	.085	-.336	.158	-.211	.328	-.150	-.157	1	-.232	.460**
N	.323	.638	.056	.380	.237	.062	.405	.383	.33	.193	.007
	33	33	33	33	33	33	33	33	33	33	33
Speed Endurance	.047	-.024	.172	.082	.081	.292	.242	.210	-.232	1	-.176
N	.795	.893	.338	.649	.654	.099	.174	.241	.193	.33	.327
	33	33	33	33	33	33	33	33	33	33	33
Cardio respiratory endurance	-.147	.025	-.055	.329	.118	.244	-.220	-.238	.460**	-.176	1
N	.415	.892	.762	.061	.513	.171	.219	.183	.007	.327	.33
	33	33	33	33	33	33	33	33	33	33	33

*. Correlation is significant at the 0.05 level

TABLE 7. The relation between the ACTN3 RX genotype and the physiological and physical variables

Dependent Variable	Concentration of ACTN3 Gene	ACTN3 Gene	Pulse Rate	Aerobic capacity	Anaerobic capacity	Vital Capacity	Lactic acid	Speed 30 m	Vertical jump	Speed Endurance	Cardio respiratory endurance
Concentration of ACTN3 Gene	1	-.029	.278	.224	.154	.067	-.463*	-.167	-.470*	.111	-.056
N	28	.885	.152	.252	.434	.735	.013	.395	.012	.573	.778
Size of ACTN3 Gene	-.029	1	.153	-.197	-.229	-.158	-.143	.121	-.213	.004	-.270
N	.885	.885	.437	.315	.242	.420	.467	.540	.276	.985	.165
	28	28	28	28	28	28	28	28	28	28	28
Pulse Rate	.278	.153	1	.114	.068	-.462*	-.243	.168	-.210	-.054	-.018
N	.152	.437	.28	.564	.729	.013	.213	.391	.283	.786	.926
	28	28	28	28	28	28	28	28	28	28	28
Aerobic capacity	.224	-.197	.114	1	-.103	.100	.128	.070	-.258	-.210	-.078
N	.252	.315	.564	.28	.604	.611	.517	.724	.185	.284	.693
	28	28	28	28	28	28	28	28	28	28	28
Anaerobic capacity	.154	-.229	.068	-.103	1	.074	-.420*	-.247	-.236	.037	.153
N	.434	.242	.729	.604	.28	.708	.026	.205	.226	.850	.438
	28	28	28	28	28	28	28	28	28	28	28
Vital Capacity	.067	-.158	-.462*	.100	.074	1	-.112	-.309	-.193	-.231	-.133
N	.735	.420	.013	.611	.708	.28	.569	.109	.325	.236	.501
	28	28	28	28	28	28	28	28	28	28	28
Lactic acid	-.463*	-.143	-.243	.128	-.420*	-.112	1	.002	.367	.240	.029
N	.013	.467	.213	.517	.026	.569	.28	.992	.055	.219	.886
	28	28	28	28	28	28	28	28	28	28	28

Speed 30 m		-.167	.121	.168	.070	-.247	-.309	.002	1	.189	-.167	-.113
		.395	.540	.391	.724	.205	.109	.992		.334	.394	.569
	N	28	28	28	28	28	28	28	28	28	28	28
Vertical jump		-.470*	-.213	-.210	-.258	-.236	-.193	.367	.189	1	.073	.047
		.012	.276	.283	.185	.226	.325	.055	.334		.711	.812
	N	28	28	28	28	28	28	28	28	28	28	28
Speed Endurance		.111	.004	-.054	-.210	.037	-.231	.240	-.167	.073	1	-.008
		.573	.985	.786	.284	.850	.236	.219	.394	.711		.969
	N	28	28	28	28	28	28	28	28	28	28	28
Cardio respiratory endurance		-.056	-.270	-.018	-.078	.153	-.133	.029	-.113	.047	-.008	1
		.778	.165	.926	.693	.438	.501	.886	.569	.812	.969	
	N	28	28	28	28	28	28	28	28	28	28	28

*. Correlation is significant at the 0.05 level

Tables 6 and 7 indicate that there is a statistically significant correlation between the concentration of ACTN3 RR and RX genotypes and the rate of lactic acid in the blood as the two genotypes are associated with the existence of a large percentage of the white muscle fibers of the research sample subjects. It is also clear from these two tables that there is a non-statistically significant correlation between the concentration of ACTN3 RR and RX genotype and the rest physiological and physical variables under consideration (Pulse Comfort- Aerobic Capacity- Anaerobic Capacity- Vital Capacity- Power - Speed 30 m – speed endurance - Cardio respiratory endurance). This non-statistically significant correlation may be due to the smallness of the research sample size and the training of the research sample subjects in Albaha (2270 m above sea level).

Discussion

This study mainly concentrated on describing of the relation between ACTN3 gene polymorphism distribution and some physiological and physical variable in the high altitude specifically Albaha provenance (2270 m above the sea level). A sample of 68 Saudi healthy male soccer players, were enrolled where 48.53% of the subjects represent the RR genotype, 41.18% represent the RX genotype and 10.29% represent the XX genotype.

Through analysing the players performance in soccer, the player performs periodically in different intensities (low- moderate – high) based on his position in field, playing methods, opponent performance style and the diversity of situations through training and competitions. So it is preferred through the selection of the sports activities players that depend on power and speed and their components to limit the selection in the RR and RX genotypes subjects where the counterpart R is the basis for the selection of sports players that rely on power and speed. In contrast, through the selection of the sports activities players that depend on endurance, one copy or two of the X's must be available.

Although the XX genotype is characterized by endurance for long periods, it may not match the modern soccer. Where the results of the study indicate the existence of seven subjects of the sample having the XX genotype and therefore we must confirm on the importance of the skill an essential factor in soccer players selection, that should not be neglected besides the scientific quantification of the training loads for these players as well as the proper guidance in selecting the suitable playing centres that commensurate with their biological abilities to benefit from their high skill abilities in the exercise.

Tables 4 and 5 clarify that there are statistically significant differences between the three ACTN3 genotypes in (anaerobic capacity-Lactic acid-speed 30 m-power) for the RR genotype. The researchers due this to the great role of the ACTN3 gene which is largely formed in the RR, in generating power in high-speed and the ability of the individual to adapt to practice training strongly and in proportion to the requirements of the championship. Therefore, the genetic readiness saves a lot of time, effort and money through testing individuals who are well prepared for activities that require a great deal of muscle strength.

The results indicate that the concentration of lactic acid in the blood is higher for RR genotype than RX genotype. The researchers due this to the ability of muscles of the subjects having RR genotype, which is characterized by the presence of white muscle fibers compared to the RX and XX genotypes as the lactate produced by the rapid white muscle fibers is transferred to slow red adjacent fibers for use in energy production (Potocka et.al, 2019). The slow red fibers have a greater ability to use lactic acid as fuel for energy production and lactate transfer method between muscle cells is spread or transport and as if this system is controlled, the appearance of fatigue can be delayed and the physical performance period can be increased (Ghosh, 2004; Ishii & Nishida, 2013).

And as the sample subjects exist at high altitude (2270m), this contributed to the increase of anaerobic capabilities physiological and physical variables, as the training in altitude positively affect short-distance competitions, jump and throw. It is scientifically proven that the higher the level of the earth the lower the gravity, the intensity of the air and the air resistance, which positively affects the acceleration process in short distances sprint and also in the activities that need high power and speed.

There are statistically significant differences between the three genotypes in (Endurance Speed) for RX genotype. Also statistically significant differences are found between the three genotypes in (pulse comfort - aerobic capacity - vital capacity - Cardio respiratory endurance) for XX genotype, as the R577X gene is essential

for the slow and effective muscle contraction for endurance athletes, and the Lack of active ACTN3 benefits the slow muscle performance in endurance races (MacArthur& North , 2007; Garton et. al, 2018; Del Coso et. al, 2018).

The results indicate a high pulse rate in the RR and RX genotypes subjects due to the high altitude they live in, exhibited to the lack of atmospheric pressure, oxygen pressure, humidity and the increased coldness, blood pressure, pulmonary arterial pressure which may be up to three times the normal level, also the accelerated heartbeat, and temporary change in hemoglobin's ability to carry oxygen.

While for the XX genotype a low heart rate during resting is detected as the subjects of this genotype don't have the ACTN3 protein because their muscle fibers mostly are of the slow type and therefore they have ACTN2. And thus their aerobic capabilities improve and their heart chambers expand which have a positive impact on increasing the beating time and thus the heartbeats decrease.

The results indicate that there are statistically significant differences in anaerobic capacity (anaerobic capacity - speed - speed endurance - power) between the three genotypes of the ACTN3 for the RR and RX. There are also statistically significant differences in aerobic capacity (pulse comfort- Vo_2 max -vital capacity-power) between the three genotypes of the ACTN3 gene for the XX genotype which, confirms the correlation of the RR and RX genotypes with the activities characterized by speed and power and the XX genotype with the endurance activities.

Tables 6 and 7 indicate that there is a statistically significant correlation between the concentration of ACTN3 RR and RX genotype and the rate of lactic acid in the blood as the two genotypes are associated with the existence of a large percentage of the white muscle fibers of the research sample subjects. While the white and red muscle fibers are related to MCT4 gene, as MCT4 gene has greater ability to transfer lactic from muscle to blood, to generate more muscular energy during violent training.

It is also clear from these two tables that there is a non-statistically significant correlation between the concentration of ACTN3 RR and RX genotype and the rest physiological and physical variables under consideration (Pulse Comfort-Aerobic Capacity-Anaerobic Capacity- Vital Capacity- Power - Speed 30 m - speed endurance - Cardio respiratory endurance). This non-statistically significant correlation may be due to the smallness of the research sample size and the existence of the research sample subjects in Albaha (2270 m above sea level), that led to some physiological changes to the players differing from these physiological changes at the sea level, due to the biological adaptation of the altitude and the ability to perform.

Conclusions

This study presents the first study to investigate the relation between the ACTN3 gene polymorphism distribution in high altitude (2270 m above sea level) and some physical and physiological variables as a genetic marker for selecting soccer players. The results indicate the existence of significant differences in the ACTN3 gene polymorphism distribution (RR, RX and XX), also the existence of a correlation between the ACTN3 gene polymorphism distribution and some physical and physiological variables for the RR followed by the RX. While the ACTN3 XX genotype has showed a correlation with the aerobic ability variable, indicating its importance in sports requiring endurance for long periods, and hence the researchers confirm on the importance of the skills as a major factor in soccer players' selection besides the proper guidance in selecting the suitable playing centers and the ACTN3 gene polymorphism distribution. Finally the researchers recommend that, similar comparative studies of different biological environments (high altitude- sea level) and their impact on players' biological factors and performance in different sports activities should be conducted. Also the components of the training loads should be distributed and quantified especially (intensity - size) according to the biological environment to avoid health risks the players may be exposed to.

Conflicts of interest

No potential conflict of interest was reported by the authors. The present study complies with the current laws of the country in which it was performed.

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