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REVIEW

microRNA-mediated cardiac remodeling in athletes

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It has been conclusively proven that physical activity exerts beneficial effects on individual health. However, endurance activities in susceptible individuals can increase the risk of concerning cardiovascular conditions such as ventricular hypertrophy or arrhythmia. This increased risk can be attributed to a cardiac remodeling process specifically associated with endurance sports. In recent years, microRNAs (miRNAs) have been postulated to play many roles in health and disease. In the heart miRNAs regulate electrical remodeling, cardiac dilatation, fibrosis, calcium handling, heart failure, atrial fibrillation and autonomic tone in myocardial infarction. A growing body of evidence suggests that miRNAs also regulate endurance sports induced remodeling of the heart. Since miRNAs circulate in the blood they have a potential role as biomarkers in athletes indicating the degree of remodeling and predicting the risk of progression to an overt disease state.

Keywords: microRNAs; biomarker; cardiac remodeling; athletes

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Introduction

MicroRNAs (miRNAs) are endogenous small non-coding RNA-sequences (~22 nucleotides long) that regulate post-transcriptional gene-expression and play important roles in many biological processes. Since their first discovery in *Caenorhabditis elegans* in 1993, miRNAs have been found to exist in almost all organisms and are largely conserved across species [1, 2]. The biogenesis of miRNAs starts when long primary miRNAs (pri-miRNAs) are transcribed in the cell nucleus and are folded into “stem-loop”-like structures. The splicing enzyme Drosha cleaves these pri-miRNAs into small hairpin-shaped precursor miRNAs (pre-miRNAs, ~70 nucleotides), which are then exported to the cytoplasm by Exportin-5 and are further cleaved by Dicer into mature miRNAs [3]. Mature miRNAs interact with the RNA-induced silencing complex (RISC) to form the miRNA-RISC complex, which binds to the specific complementary sequence of their targeted mRNAs in the 3’-untranslated region [4]. MiRNAs regulate targeted mRNA expression at the post-transcriptional stage by one of two methods. Imperfect complementary binding leads to inhibition of translation while perfect complementary binding leads to mRNA degradation.

In humans, over 2000 miRNAs are predicted to be present in the genome [5]. Extensive studies have established their importance in the healthy and diseased states of many organs, including the heart. MiRNAs regulate cardiac remodeling processes and are involved in the pathophysiology of coronary heart disease, myocardial infarction (MI), heart failure (HF) and atrial fibrillation (AF) [5, 6]. Dysregulation of miRNA expression is highly correlated with pathological cardiac conditions such as fibrosis and arrhythmias in both clinical settings and animal models [7, 8], which implicates them as potential therapeutic targets. Recently, reliable measurement of circulating miRNA levels in blood samples has led to the emergence of miRNAs as novel biomarkers for cardiovascular disease [9, 10].

In this review, our focus is to summarize the current knowledge about miRNA-mediated cardiac remodeling in athletes, and their potential impact as circulating biomarkers.

Cardiac remodeling in athletes

Physical training by athletes may impose different hemodynamic loads on the heart, depending upon the type of activity. Endurance athletes engage in isotonic or aerobic activities such as running, swimming or cycling. Resistance athletes participate in isometric or anaerobic activities such as weightlifting. At extremes of exertion there is likely to be overlap between the two categories, while some activities such as rowing have inherent overlap [11]. Prolonged exposure to high levels of physical activity leads to departures from normal cardiac structure including left ventricular hypertrophy and left atrial dilatation, which have become known collectively as "athlete's heart" [12]. Endurance training also leads to biatrial enlargement. Such structural effects are reversible with a period of detraining, a phenomenon that has use clinically in differentiating marked physiological hypertrophy from hypertrophic cardiomyopathy [13].

Both endurance and resistance training lead to an increase in overall cardiac volume. Strenuous endurance training such as long-distance running results in the greatest increase in cardiac volume in comparison to, for example, weightlifting [14]. MRI data support the hypothesis that the increase in LV mass is due to expansion of the myocyte rather than the extracellular component of the myocardium[15]. These structural changes are associated with normal or above normal cardiac function [16], although ejection fraction may appear reduced. It should be noted that the left ventricular systolic ejection fraction has a lower cut off for normal in trained athletes (45%), although stroke volume is preserved [17].

In terms of an explanation for these alterations, a number of mechanisms have been proposed. The different hemodynamic effects of endurance and resistance training have been held responsible for the development of eccentric and concentric left ventricular hypertrophy respectively [18]. Endurance training effectively results in volume overload while resistance training is analogous to pressure overload [19].

From a molecular point of view, physiological remodeling is characterized by differential activation of metabolic pathways that are distinct from those seen in pathological remodeling, in which reactivation of the fetal gene program is seen [20]. Activation of the IGF-1/IGF-1R/Akt pathway has been strongly implicated in the development of athlete's heart [21]. The protein kinase AKT1 appears to play a key role. AKT1 knockout mice fail to develop hypertrophy in response to endurance training but not pressure overload [22].

Athletic training is also associated with disturbances in electrophysiological characteristics of the heart. Physiological electrocardiographic changes are observed in the majority of trained athletes and can mimic some pathological conditions, including heart block and hypertrophic cardiomyopathy [23]. This may lead to difficulty in diagnosing those with underlying cardiovascular disease, and is of particular relevance given the risk of sudden death in athletes with concomitant cardiovascular disease [24]. As such specific recommendations for interpretation of the ECG in athletic individuals have been issued [25]. Genetic screening of athletes with abnormal ECGs revealed mutations in genes associated with hypertrophic cardiomyopathy in 5% of cases [26].

Endurance training is also well known to cause resting bradycardia. The mechanism for this is controversial, with evidence supporting increased vagal tone and also downregulation of HCN4, the gene that encodes the pacemaker ionic channel [27, 28]. Recent work suggests that bradycardia in athletes is not associated with an increased risk of arrhythmias or syncope [29].

Participation in endurance sport may increase the risk of AF significantly, although the quality of evidence supporting this observation has been questioned [30, 31]. Regular or moderate physical activity does not appear to be associated with the same increased risk, and may be protective [32, 33]. There may also be gender differences in the risk of AF associated with physical activity, with men at higher risk [34].

Table 1. Circulating miRNAs in athletes

Exercise modality	acute/chronic	miR-changes	miRNA	ref.
Cycling (60 minutes)	acute	downregulation	miR-486	[90]
Cycling (60 minutes)	acute	downregulation (immediately)	miR-106a, -221, -30b, -151-5p, let-7i, -146, -652, -151-3p	[91]
		upregulation (after 1 hour)	miR-338-3p, 330-3p, -223, -139-5p, -143	
		upregulation (3 hours later)	miR-1	
Cycling	acute	upregulation	miR-146a, -222, (-21, -221)	[92]
Marathon running	chronic	elite runners: upregulation	miR-1, miR-133a	[10]
		non-elite runners: downregulation	miR-26a	
Cycling (3d/week, 4 weeks)	chronic	downregulation	miR-486	[90]
Rowing (90 days)	chronic	upregulation	miR-20a	[92]
Cycling (5x/week, 12 weeks)	chronic	downregulation	miR-342-3p, let-7d, -766, -25, -148a, -185, -21, -103, -107	[91]
Running (13 hours/week)	chronic	upregulation	miR-222, -21, -146a, -221	[94]

The cause for the increased risk in endurance athletes is unknown. Potential mechanisms include inflammation and altered vagal tone [12, 35, 36]. Increased left atrial size has also been observed in approximately 20% of athletes, although this enlargement is not accompanied by electrical abnormalities as reflected by the appearance of the P wave on 12-lead ECG [37, 38]. In general progressively larger atria are associated with an increased risk of atrial fibrillation [39]. The largest increases in atrial size are seen in endurance athletes as compared to strength trained athletes [40].

It should be noted that despite the above listed departures from normality, a review of mortality data pertaining to endurance training and cardiac remodeling by Scharhag *et al*. found that endurance activities are associated with significant increases in longevity and reductions in mortality[14]. Given the overlap between athlete's heart and some disease states therefore, concerted efforts have been made to define precisely the electrocardiographic and imaging parameters that best discriminate between those with a healthy cardiac substrate and those with underlying disease [41, 42].

MiRNAs and cardiac remodeling

Many cardiac diseases, from genetic to acquired, have cardiac remodeling in common, irrespective of the underlying cause. Remodeling is a complex process; the term serves as an umbrella that includes electrical remodeling, structural remodeling, Ca^{2+}-handling abnormalities, and neurohormonal dysregulation [6]. Electrical remodeling involves altered expression or function of ion channels, which can result in changes of the generation and conduction of electrical pulses in the heart, therefore facilitating arrhythmogenesis. Structural remodeling of the myocardium refers to tissue fibrosis or enlargement of atria or ventricles, providing an anatomical substrate for arrhythmia. Abnormal calcium handling in the cardiomyocyte undermines intracellular calcium homeostasis and can result in afterdepolarizations [43,44]. Dysregulation of the autonomic nervous system, such as altered vagal or sympathetic tone, can have downstream effects on ion channel function or cause intracellular calcium overload [45]. The role of miRNAs in cardiovascular disease has been intensively investigated and it is well recognized that they play a significant role in the cardiac remodeling process.

The miRNA transcriptome has distinctive expression profiles among different tissues including the heart [46, 47]. A subset of known miRNAs are strongly expressed in the normal heart [48]. The expression of these cardiac-enriched miRNAs is significantly changed in the process of cardiac disease development [48]. One key aspect of miRNA biology is that one or a group of similarly expressed miRNAs can regulate multiple steps in a complex physiological process. Therefore, miRNAs whose expression is found to be tightly associated with cardiac remodeling have great therapeutic potential. Table 1 shows miRNAs that are involved in cardiac remodeling.

MiRNAs known to be involved in cardiac electrical remodeling include miR-1, miR-133, miR-26, miR-208a, miR-328 and miR-499. MiR-1 and miR-133 are cardiac- and skeletal muscle specific miRNAs. MiR-1 targets *KCNJ2*, *GJA1*, *KCNE1* and *KCNB2* expression in the heart. Downregulation of miR-1 and upregulation of Kir2.1 (encoded by *KCNJ2*) was observed in the heart of patients with AF, which leads to increased I_{K1} and shortened atrial action potential duration (APD), alterations that promote AF [49]. On the other hand, upregulation of miR-1 has been reported in the tachypaced rabbit atrium and the ventricles of rat, guinea pig, dog and human with MI or HF, accompanied by reduced Kir2.1, connexin-43 (encoded by *GJA1*), KCNE1 and KCNB2 expression, which can result in APD prolongation. This can in turn cause early afterdepolarization (EAD) and conduction slowing, both of which are also substrates for arrhythmogenesis [50-52]. MiR-133 targets ERG. Upregulation of miR-133 with ERG downregulation is seen in the guinea pig heart with nicotine-induced atrial remodeling, leading to APD prolongation and EADs [52]. MiR-26 also targets *KCNJ2*. Downregulation of miR-26 and upregulation of Kir2.1 were found in the atrial tissue of dogs with HF and patients with AF [53]. MiR-208 targets *GJA5* (encoding connexin-40) in the heart and overexpression of mir-208a, an isoform found in the adult heart, causes connexin-40 downregulation. This alters electrical conduction in the mouse heart and increases vulnerability to

arrhythmia [54]. MiR-328 targets both α (*CACNA1C*) and β (*CACNB1*) subunits of the L-type calcium channel. MiR-328 negatively regulates L-type calcium channel expression; dysregulation can alter APD, predisposing the canine heart to arrhythmogenesis [55]. MiR-499 is found to target *KCNN3* from in vitro studies. In human AF patients, its expression was increased in atrial tissue and SK3 (encoded by *KCNN3*) protein level was reduced, suggesting its role in cardiac electrical remodeling [56].

Several miRNAs - miR-21, miR-26, miR-29, miR-30, miR-133, miR-499 and miR-590 - participate in cardiac structural remodeling. They target genes encoding proteins involved in extracellular matrix turnover and pro- or antifibrotic signaling cascades in the heart. MiR-21 is mainly expressed in cardiac fibroblasts and targets *spry1* that encodes sprouty 1, an antifibrotic regulator of the profibrotic ERK-MAP kinase pathway [57]. Upregulated miR-21 expression is a consistent finding among HF models in mouse, rat, dog and pig, and also human atrial tissue from AF patients [57-62]. Decreased sprouty 1 expression, increased expression of profibrotic connective tissue growth factor (CTGF), lysyl oxidase and Rac1-GTPase, and increased cardiac fibrosis, due to increased synthesis of extracellular matrix proteins such as collagen-1, collagen-3, fibrillin and matrix metalloproteinase 2 (MMP2), are reported in many of these studies [57, 59, 62]. The transcription factor phosphatase and tensin homologue (PTEN) is also a target of miR-21. A murine model of MI found increased levels of miR-21 and downregulation of PTEN, resulting in increased synthesis of MMP2 [58]. MiR-26 is involved in cardiac structural remodeling through targeting transient receptor potential cation 3 (TRPC3) channels in cardiac fibroblasts [63]. Downregulation of miR-26 and upregulation of TRPC3 expression with increased fibroblast proliferation were observed in a canine ventricular tachy-pacing model [63]. Downregulation of miR-29b is associated with increased collagen, fibrillin, elastin and fibrosis in animal models of cardiac hypertrophy or HF, and in AF patients [64-66]. MiR-133 and miR-30 target CTGF. They were found to be downregulated, whereas CTGF expression and cardiac fibrosis were increased in different animal models and human cardiac tissue with cardiac hypertrophy [52, 61, 66-68]. MiR-499, which alters collagen content through targeting calcineurin, was found to be downregulated in a murine model of MI [69]. MiR-590 can target the profibrotic mediator transforming growth factor β receptor-II (*TGF- βRII*), and its downregulation is associated with increased *TGF- βRII* and collagen content in *in vitro/in vivo* studies of the canine heart, and with increased atrial fibrosis in human AF patients [52].

MiRNAs found to regulate cardiac calcium handling are miR-1, miR-214, miR-574-3p, miR-24 and miR-22 [44]. MiR-1 targets B56α, a regulatory subunit of PP2A. Upregulation of miR-1 represses PP2A activity by downregulating B56α, and therefore increases cardiac sarcoplasmic reticulum (SR) calcium-induced calcium release channel (CICR) phosphorylation, resulting in increased spontaneous SR calcium release, facilitating delayed afterdepolarizations (DAD)[70]. MiR-1 also targets *NCX1*, encoding the sodium-calcium exchanger. In a HF model, decreased miR-1 and increased NCX1 were reported [71]. MiR-214 can target NCX1 and is upregulated in cardiac hypertrophy and HF [72]. MiR-574-3p is upregulated in ventricular tissue from MI patients [73]. It targets SERCA2a. Decreased SERCA2a protein was found in the infarcted area, which decreases Ca^{2+} removal from cytoplasm and might cause Ca^{2+} overload in MI [73]. MiR-24 affects calcium handling through targeting junctophilin 2 (JP2), a protein ensuring proper T-tubule-SR structural coupling [74]. MiR-22 regulates SERCA2a expression through targeting purine-rich element-binding protein B (PURB) that represses SRF [75]. SRF controls SERCA2a expression. Altered miR-22 levels have been found in human and animal models of HF [76, 77], in which SERCA2a is reduced, leading to disrupted cardiac function.

The role of miRNAs in dysregulation of the autonomic nervous system has been less fully explored. MiR-133 reportedly controls multiple components of the β1AR transduction cascade [78]. Increased miR-133 expression attenuates apoptosis and reduces fibrosis in the transaortic constriction mouse model that is used to study left ventricular hypertrophy [78]. Renal sympathetic denervation upregulates miR-101a and -133a and downregulates miR-21, therefore reducing connective tissue growth factor (CTGF) and accompanied by decreased plasma norepinephrine, renin, angiotensin II and aldosterone levels, ameliorating post-MI LV fibrosis [79].

MiRNAs are relatively stable and detectable in the blood [80]. Several mechanisms have been proposed for the intra- to extra-cellular transposition of miRNAs as shown in Figure 1 [7]. MiRNAs can be packed in exosomes, microvesicles or apoptotic bodies and secreted [10, 81]. They can also be released from dead cells in severely damaged myocardium

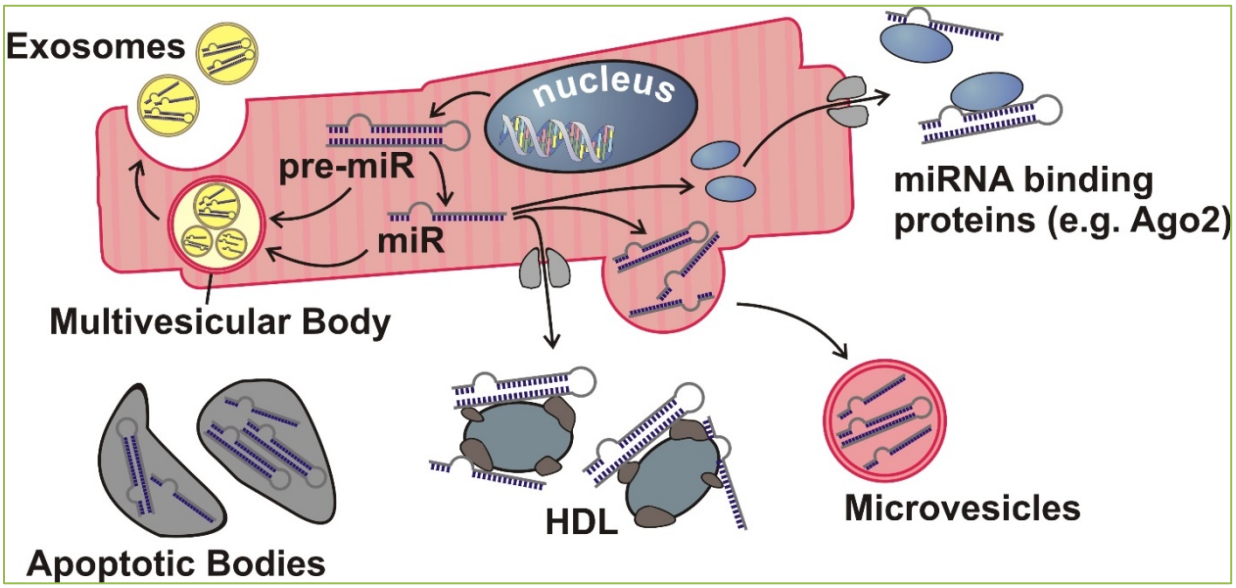

Figure 1. miRNA release into circulation. MiRNAs are released into the circulation by various mechanisms using several carriers including exosomes, microvesicles, apoptotic bodies, HDL, or other miRNA binding proteins.

such as in MI [9, 82]. These properties flag miRNAs as potential biomarkers for cardiovascular disease [7, 82]. Over the past five years, emerging studies have evaluated the diagnostic and prognostic potential of circulating miRNAs in patients with diseases like AF and/or HF [7]. For example, as mentioned earlier, miR-29b and miR-21 are involved in cardiac structural remodeling through the regulation of profibrotic protein expression. Plasma miR-29b levels were found to be significantly reduced in patients with AF or HF [65]. Plasma miR-21 as well as miR-150 levels were significantly reduced in AF patients from the miRhythm study [83].

Interestingly, our group reported that changes in several circulating mRNA levels correlated with atrial remodeling in athletes [10]. Although promising, available data on the evaluation of circulating miRNAs as biomarkers in clinical practice reveals huge variety and discrepancy of miRNA changes in patients with AF or HF [7]. The reliability of miRNAs as biomarkers for diagnosis and prognosis in cardiovascular diseases requires further future research.

miRNAs in athletes

There are limited data regarding miRNA biology that is specific to the athlete. A detailed study examining the profile of selected miRNAs (1, 26a, 29b, 30a and 133a) in elite and non-elite runners at different time points surrounding pre-marathon training and running a marathon revealed differential temporal patterns between the two groups. MiR-1 and -133a increased significantly after the marathon in the ER group only, while miR-26a decreased significantly in the ER group only [10]. These findings were correlated with echocardiographic indices of left atrial size.

MiRNAs have been proposed as potentially useful biomarkers in the field of anti-doping as part of the so-called biological passport [84]. Different miRNAs can be used to detect treatment with erythropoietin stimulating agents, recombinant human growth hormone, autologous blood transfusion and possibly testosterone [84-87]. Use of miRNAs as biomarkers in athletes is complicated by a number of issues. Athletic activity results in hemolysis which can confound measurement of plasma miRNAs due to the release of miRNAs from red blood cells [88]. Blondal *et al* suggest a comparison of miRNA-451 and -23a may be useful as a miRNA-expression based indicator of hemolysis [89].

In addition, acute and chronic exercise itself impacts the expression profile of several miRNAs. For example, Aoi *et al* noted a decrease in the plasma level of miRNA-486 following exercise [90]. Nielsen *et al* observed downregulation of 8 miRNAs (miRNA-106a, -221, -30b, -151-5p, let-7i, miRNA-146, -652 and -151-3p) immediately following a bout of exercise, but conversely an increased plasma level of 5 miRNAs one hour later (miRNA-338-3p, -330-3p, -223, -139-5p and -143) and an increased plasma level of only one at three hours following exercise (miR-1) [91]. In contrast to these data, Baggish *et al* observed an upregulation of 4 miRNAs (miRNA-146a, -222, -21 and -221) immediately following a bout of exercise, with recovery of levels to baseline after an hour [92].

Following chronic training, Nielsen at al observed a different expression profile, with downregulation of 7 miRNAs (miR-342-3p, let-7d, miR-766, miR-25, miR-148a,

miR-185 and miR-21) and upregulation of 2(miR-103 and miR-107) [91]. Consistent with their acute exercise data however, Baggish *et al* observed significantly elevated levels of 4 miRNAs miRNA-146a, -222, -21 and -221) [92]. The contradictory findings pertaining to miRNA-21 are of particular interest, as lower levels of miRNA-21 have been implicated in a variety of cardiac pathologies including ventricular hypertrophy, heart failure and ischemia [93].

Finally, different modalities of athletic activity result in differential circulating miRNA profiles [94]. Four miRNAs (miR-222, miR-21, miR-146a and miR-221) were found to be differentially expressed depending on whether the athlete engaged in endurance or strength training.

Therefore, in addition to correcting for hemolysis, the type of activity, quantity of training and timing of sampling would need to be carefully recorded and considered as a possible confounder in the assessment of miRNAs in these populations.

Conclusion and future perspective

MiRNAs are measureable and show cardiac disease-specific expression profiles. They may play a crucial role in the pathophysiology of cardiovascular illness. They show promise as biomarkers and may play a role in the development of novel therapies in the future, either as drug targets or predictors of response. They participate in the multiple complex biological processes of cardiac remodeling, many of the features of which are also present in athlete's heart. Circulating miRNAs may therefore serve as biomarkers for cardiac remodeling in athletes. However, future research is needed to investigate how miRNAs are regulated by exercise and their roles in the cardiac adaptation to physical training. Our improved understanding of miRNA-mediated cardiac remodeling in athletes can help to manage and prevent exercise-training induced cardiac diseases.

Conflicting interests

The authors have declared that no conflict of interests exist.

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Abbreviations

AF: atrial fibrillation; Akt1: AKT serine/threonine kinase 1; APD: action potential duration; CACNA1C: calcium voltage-gated channel subunit alpha1 C; CACNB1: calcium voltage-gated channel auxiliary subunit beta 1; CICR: calcium induced calcium release; CTGF: connective tissue growth factor; DAD: delayed afterdepolarization; EAD: early afterdepolarization; ECG: electrocardiography; ER: elite runners; ERG: Ether-à-go-go; ERK: extracellular signal-regulated kinase; GJA1: gap junction protein alpha 1; GJA5: gap junction protein alpha 5; GTPase: Guanosine-5'-triphosphatase; HCN4: hyperpolarization activated cyclic nucleotide gated potassium channel 4; HDL: high density lipoprotein; HF: heart failure; IGF-1: Insulin-like growth factor 1; IGF-1R: Insulin-like growth factor 1 receptor; JP2: junctophilin 2; KCNB2: potassium voltage-gated channel subfamily B member 2; KCNE1: potassium voltage-gated channel subfamily E regulatory subunit 1; KCNJ2: potassium voltage-gated channel subfamily J member 2; KCNN3: potassium calcium-activated channel subfamily N member 3; LA: left atrium; LV: left ventricle; MAP: Mitogen-activated protein; MI: myocardial infarction; miRNA: microRNA; MMP2: matrix metalloproteinase 2; mRNA: messenger RNA; NCX1: Na+/Ca2+ exchanger; NER: non elite runners; PP2A, Protein phosphatase 2; PTEN: phosphatase and tensin homologue; PURB: purine-rich element-binding protein B; RISC: RNA-induced silencing complex; RNA: ribonucleic acid; SERCA1a: sarcoplasmatic Ca2+-ATPase; SK3: small conductance calcium-activated potassium channel 3; spry1: sprout 1; SR: sarcoplasmatic reticulum; TGF- βRII: transforming growth factor β receptor-II; TRPC3: transient receptor potential cation 3 channel.

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