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Genetics of Muscle Stiffness, Muscle Elasticity and Explosive Strength

by
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Muscle stiffness, muscle elasticity and explosive strength are the main components of athletes' performance and they show a sex-based as well as ethnicity variation. Muscle stiffness is thought to be one of the risk factors associated with sports injuries and is less common in females than in males. These observations may be explained by circulating levels of sex hormones and their specific receptors. It has been shown that higher levels of estrogen are associated with lower muscle stiffness responsible for suppression of collagen synthesis. It is thought that these properties, at least in part, depend on genetic factors. Particularly, the gene encoding estrogen receptor 1 (ESR1) is one of the candidates that may be associated with muscle stiffness. Muscle elasticity increases with aging and there is evidence suggesting that titin (encoded by the TTN gene), a protein that is expressed in cardiac and skeletal muscles, is one of the factors responsible for elastic properties of the muscles. Mutations in the TTN gene result in some types of muscular dystrophy or cardiomyopathy. In this context, TTN may be regarded as a promising candidate for studying the elastic properties of muscles in athletes. The physiological background of explosive strength depends not only on the muscle architecture and muscle fiber composition, but also on the central nervous system and functionality of neuromuscular units. These properties are, at least partly, genetically determined. In this context, the ACTN3 gene code for α-actinin 3 has been widely researched.

Key words: *sports genetics, genetic markers, genetic polymorphism, muscle rigidness, power.*

Introduction

Competitive athletes are constantly subjected to extreme training loads, attempting to optimize their adaptive potential, and as a consequence reach the best possible sport results. However, such exploitation of the body leads to chronic fatigue, stress, and thus, increases the risk of injury. In professional athletes, muscle injuries are the most common trauma, their incidence increases especially for sprinters and jumpers (Opar et al., 2012). The most frequent cause of such injuries is the loss of joint flexibility and muscle stiffness which is one of the main components of joint flexibility (Miyamoto et al., 2017; Watsford et al., 2010). Most often, injuries occur while exercising and training, as confirmed by both morphological evidence and loss of strength. Swelling and the release of intracellular enzymes as well as muscle pain caused by muscle stiffness are very common.

Muscle stiffness is described as the ratio between changes in force and muscle deformation (Rieder et al., 2015). Muscle performance is affected when muscle's stiffness is increased and in consequence such muscle requires more force to deform than before. Stiffness depends on the muscle structure (length and cross-sectional area), forces applied and intrinsic material properties of

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muscle (Baumgart et al., 2000). According to the majority of research on biomechanical properties of muscle, higher stiffness is favourable for fast stretch shortening cycle activities and activities characterized by high movement velocity (Brughelli et al., 2008; Bret et al., 2002). Stiffness may also have significant implications in force production within muscles (Pożarowszczyk et al., 2018).

Recently published research suggests that the duration of muscle contraction can provoke changes in the elasticity of tendon structures. Scientific research confirms that important factors in reducing joint flexibility and deterioration of muscle functions are age and sex, while genetic polymorphisms are rather associated with muscle stiffness (Kocur et al., 2019; Kumagai et al., 2019).

Elasticity is a structural adaptation of tendons and fascia, which is a specific property of the human musculoskeletal system that determines its ability to perform a motor task in the full amplitude of the existing range of motion. It is an extremely important element affecting the development of strength and power output of athletes. Moreover, a crucial feature for every athlete is a high level of kinetic energy during the interaction between two objects (elastic collision). Muscle and tendon mechanical properties along with the elastic properties of the joint structures and stiffness arising from muscle actions affect the stiffness of the whole limb. The conversion of energy to motion involves recoil of some elastic energy in muscles and tendons. This is why a "stiffer" muscle is more effective at an economic energy transfer with lower oxygen uptake (Dumke et al., 2010).

Many studies have confirmed important statistical relationships between high levels of lower-body strength as well as explosiveness and athletic performance (Krzysztofik et al., 2020; Suchomel et al., 2015; Wang et al., 2012). In diverse sport disciplines such as track & field sprints, throws, jumps and hurdling, as well as most team sports and combat sports, the rate of power development has a great impact on performance. There are other key factors influencing high performance in the above-mentioned sport disciplines and explosive strength is one of them, apart from anthropometric characteristics and proper technique (Gołaś et al., 2016; Maszczyk et al., 2020). The basic role of explosive strength is to enable muscles to generate maximum strength in the shortest time possible. The amount of strength developed depends on the excitability of the nervous system and the speed of impulse transmission to the muscles, the number of recruited (stimulated) motor units, strength and speed of contraction as well as the contractibility of the stimulated muscles.

It is commonly known that rapid force generation is mainly related to the specific body build, however, the influence of the nervous system and bone structure of particular joints is still to be researched. On the other hand, scientists confirm that explosiveness, which is dependent on muscle architecture and fibre composition of the body, is genetically determined.

The role played by genetic factors in many features of athletic performance has been discussed in greater depth worldwide. However, there is still a gap in the knowledge on genetic background of such important components of athletes' performance as muscle stiffness, muscle elasticity and explosive strength. Therefore, this review mainly focuses on the distinguished variants of candidate genes that are the most promising genetic markers involved in the development of the aforementioned properties of skeletal muscles.

***ESR1* gene**

As it was mentioned before, increased muscle stiffness, defined as the load elongation characteristics of the muscle distal to the myotendinous junction, is one of the main risk factors often associated with sports-related injures (Magnusson et al., 1997; Miyamoto et al., 2017). It is commonly known that there are significant gender differences in muscle stiffness, i.e., men exhibit higher muscle stiffness than women (Morse, 2011). One possible explanation of this observation includes different levels of circulating sex hormones such as estrogen (Lemoine et al., 2003). Accordingly, some previous studies have shown that higher estrogen levels, due to suppressing total collagen (specifically, collagen types I and IV) synthesis, are associated with lower muscle stiffness in females (Kwan et al., 1996; Morse, 2011). Additionally, estrogen reduces muscle damage and accelerates muscle regeneration after injury by anti-inflammatory

and antioxidant impact on skeletal muscles (Feng et al., 2004; Tiidus, 2005).

The biological effects of estrogen are mediated via two estrogen receptors (ERs), which belong to the superfamily of nuclear receptors and family of steroid receptors that act as ligand-activated transcription factors (Wiik et al., 2009). Kumagai et al. (2019) hypothesized that gene encoding estrogen receptor 1 (*ESR1*) was one of the promising candidates that may be associated with muscle stiffness (Kumagai et al., 2019). The human *ESR1* gene is more than 140 kb long and is located in the long arm of chromosome 6 (6q25.1-q25.2). It consists of eight exons, and the position of its introns has been highly conserved between species with the exception of the 5′-end (Kos et al., 2001; Ponglikitmongkol et al.,1988).

Two functional polymorphisms in the first intron of the *ESR1* gene (rs2234693 C/T and rs9340799 G/A) may have a significant role in muscle stiffness variations among individuals (Kumagai et al., 2019). The rs2234693 C and the rs9340799 G alleles have been linked to increased gene expression and more beneficial estrogen-induced effects (Herrington et al., 2002).

These observations, together with the assumption of the estrogen's role in decreasing muscle stiffness, may lead to the suggestion that the presence of *ESR1* rs2234693 C and rs9340799 G alleles might be associated with lower muscle stiffness. This hypothesis was partially confirmed by the study of Kumagai et al. (2019). The authors examined the relationship between the two mentioned above polymorphisms in the *ESR1* gene and the history of muscle injury of 1311 top-level Japanese athletes. The study also involved the analysis of these polymorphisms as determinants of stiffness of the hamstring muscles assessed with the use of the Elastography method in 261 physically active participants. The results confirmed that the *ESR1* rs2234693 polymorphism was associated with muscle stiffness and injury susceptibility. The authors showed that the C allele, in contrast to the T allele, had a protective effect against muscle injury in athletes and it resulted in lower muscle stiffness in physically active participants. However, the role of the second rs9340799 polymorphism in occurrence of injury and muscle stiffness was not confirmed (Kumagai et al., 2019). In the future, more experimental studies are needed to confirm the relationship between the *ESR1* gene and muscle stiffness.

***COL5A1* gene**

Research has suggested that collagens, structurally and functionally a heterogeneous superfamily of proteins located in the extracellular matrix (ECM) of almost all tissues, also influence muscle stiffness, joint flexibility, and are associated with the muscle injury (Kadler et al., 2007; Miyamoto-Mikami et al., 2019). Specifically, type V collagen, i.e., a minor fibrillar collagen, is a significant structural component of connective tissue. It is essential for fibrillation of types I and III collagen, and consequently for optimal fibrillary formation and tissue quality (Collins and Posthumus, 2011).

Accordingly, the collagen type V α1 chain gene (*COL5A1*), usually associated with susceptibility to the rupture of the anterior cruciate ligament (ACL) and Achilles tendinopathy (Collins and Posthumus, 2011; O'Connell et al., 2015), is also a promising candidate gene variants of which may not only be recognized as muscle injury risk factors, but also affect muscle stiffness and joint flexibility (Miyamoto-Mikami et al., 2019).

The *COL5A1* gene spans at least 750 kb, is located on the long arm of chromosome 9 (9q34.3), and shows complex structure, having 66 exons (Takahara et al., 1995). One of the most investigated polymorphisms in this gene is the C/T polymorphism (rs12722) in the 3′-untranslated region (UTR). The rs12722 T allele exhibited enhanced mRNA stability compared to the C allele, which is associated with higher type V collagen production as well as structural and architectural changes of the collagen fibril, and as a result with general change in the musculoskeletal soft tissue mechanical properties (Collins and Posthumus, 2011).

Miyamoto-Mikami et al. (2019) examined whether the C/T polymorphism in the *COL5A1* gene is associated with joint flexibility and passive muscle stiffness in 1559 Japanese athletes with the muscle injury history and 363 healthy and physically active adults. The authors did not support the hypothesis that the rs12722 polymorphism was related to passive muscle stiffness, joint flexibility, and muscle injury in the Japanese population. However, further research is needed to establish whether the variants located

in the *COL5A1* and in other collagen genes are engaged in the change of muscle stiffness as well as the susceptibility to injury (Miyamoto-Mikami et al., 2019).

Skeletal muscle channels genes (*SCN4A, CLN1*)

Abnormal muscle stiffness of skeletal and smooth muscles is also often characterized as a clinical symptom of numerous disorders, such as myotonias (Pechmann et al., 2019; Sahin et al., 2018). Genes' polymorphisms described in the diseases are also candidate markers for increased muscle stiffness and as a consequence for sports-related injury risk in healthy and physically active individuals.

The frequent reason of these disorders are two different types of channel dysfunction: chloride (Cl) channel dysfunction and sodium (Na) channel dysfunction. Therefore, skeletal muscle sodium (*SCN4A*) or chloride (*CLN1*) channel genes seem to be the most promising muscle stiffness genetic markers (Pechmann et al., 2019). Pechmann et al. (2019) suggested that the specific genotypes were not pathogenic, however, they might modify the phenotype, and probably influence muscle physiology in healthy individuals (Pechmann et al., 2019).

***ACTN3* gene**

The α-actinins belong to the diverse superfamily of cytoskeletal proteins, named spectrins, which also includes dystrophins. The α-actinins are actin-binding proteins with multiple roles in different cell types, particularly they play a key role in the maintenance and regulation of the cytoskeleton. In the human genome, there are four genes that code for α-actinins: two non-muscle (*ACTN1* and *ACTN4*) and two muscle (*ACTN2* and *ACTN3*) (Beggs et al., 1992; Blanchard et al., 1989). The later genes code for isoforms α-actinin-2 and α-actinin-3, which act as the major component of the sarcomere contractile apparatus at the Z-line by cross-linking and anchoring actin. The α-actinin-2 is expressed in all human skeletal muscle fibers as well as cardiac muscle fibers. The expression of α-actinin-3, which is a closely related isoform (80% identical and 90% similar at the amino acid level to the α-actinin-2), is almost completely limited to the type II muscle fibers. These fast glycolytic fibers are less resistant to fatigue and eccentric damage, however, they can generate rapid and forceful contractions (Lek et al., 2010).

Considering the α-actinin-3 gene (*ACTN3*), a common single nucleotide polymorphism (SNP) commonly referred to as R577X (rs1815739), it was discovered in 1999 when searching for possible causative genes for muscular dystrophy (North et al., 1999). The SNP is a transition of Cytosine (C) to Thymine (T) in the *ACTN3* sequence, which in turn generates a premature codon stop (X) instead of the arginine codon (R) at position 577 of the α-actinin-3 amino-acid sequence. In consequence, the R577X polymorphism results in 2 alleles (R and X) and 3 genotypes (RR, RX, XX). The *ACTN3* R577 allele enables expression of a full length α-actinin-3, whereas the 577X allele abolishes α-actinin-3 production due to mRNA degradation which is mediated by the cellular nonsense-mediated mRNA decay (NMD) system (Harada et al., 2018). Homozygosity for the 577X allele (577XX genotype) results in complete deficiency of the sarcomeric α-actinin-3. The frequency of the 577X allele as well as the 577XX genotype varies between human populations from 7-9% of the 577X allele (0% of the 577XX genotype) in Sub-Saharan Africans to 43-53-59% of the 577X allele (22-25-28% of the 577XX genotype) in Europeans, Asians and native Americans, respectively (retrieved from: Database of Single Nucleotide Polymorphisms (dbSNP) 01.04.2020). The lack of functional α-actinin-3 is not detrimental to the 577XX homozygotes in terms of disease, but it alters muscle function and metabolism (Lee et al., 2016).

The report that investigated the relationship between *ACTN3* R577X SNP and athletic performance in Australian athletes was the earliest to study the consequences of the R577X polymorphism in a human population. It was shown that the RR genotype was overrepresented in sprint-power athletes, while the frequency of the XX genotype was higher in endurance athletes (Yang et al., 2003). Since that time it has been observed that 1) the XX genotype is underrepresented in power and sprint athletes and higher frequency of this genotype may be found in endurance athletes as well as 2) R allele or RR genotype frequency is significantly higher in sprint/power athletes, particularly in the group of international-level sprint/power athletes

(Eynon et al., 2009; Eynon et al., 2012; Houweling et al., 2018; Kim et al., 2014; Muniesa et al., 2010; Papadimitriou et al., 2016; Pimenta et al., 2013; Roth et al., 2008; Yang et al., 2017). Effects of the *ACTN3* R577X polymorphism on the athletes' lower-extremity power output have been measured as well. The best standing long jump, standing vertical jump as well as counter-movement jump results were registered in athletes with R allele or/and RR homozygotes rather than in α-actinin-3 deficient individuals (Orysiak et al., 2014; Yang et al., 2017). It was also observed that in the general population, the RR genotype was associated with greater muscle capacity for contractile force and that R allele carriers had greater power production when compared to the α-actinin-3 deficient individuals. Homozygous XX women produced less elbow flexor isometric strength when compared to α-actinin-3 expressing women and *ACTN3* RR men had significantly higher knee extension strength compared to α-actinin-3 deficient men (Clarkson et al., 2005a; Vincent et al., 2007). Maximal voluntary isometric contraction (MVC) torque at baseline was also greater in non-athletic RR homozygotes than in X-allele carriers (Kikuchi et al., 2017a). Taken together, these observations suggest that a strong relationship exists between the *ACTN3* genotype and human physical performance. In this line, at least one R allele increases the capacity to perform high power muscle contractions, whereas a-actinin-3 deficiency in XX homozygotes is detrimental to muscle strength and sprinting performance, both in athletes and in the general population.

An *Actn3* knockout (KO) mouse model that mimics human α-actinin-3 deficiency was generated to study the effects of *ACTN3* R577X SNP on skeletal muscle function (MacArthur et al., 2007, 2008). Despite the fact that *Actn3* KO mice were indistinguishable from the wild type (WT), they were different in muscle performance. *Actn3* KO mice produced lower grip strength, but were able to run further on a motorized treadmill before reaching exhaustion when compared to WT mice. The KO mice also showed enhanced adaptive response to endurance running and faster recovery from fatigue as well as lower muscle fiber mass, muscle strength and lower force generation. α-actinin-3 is expressed in mice exclusively in type II muscle fibers which are referred to as "fast" fibers and are responsible for rapid and forceful contractions. In particular, type IIb fibers are most prone to fatigue and rely on the anaerobic metabolism. When individual fibers of KO mice were examined, the reduction in muscle mass was observed by a specific reduction in the IIB fiber diameter. Further examination of KO mice revealed lower lactate dehydrogenase (LDH) and glycogen phosphorylase (GPh) activity which are crucial to type IIb fibers to generate force in the anaerobic pathway. Moreover, KO fast muscle fibers presented an increased activity of key enzymes in oxidative metabolic pathways such as the citric acid cycle, the mitochondrial electron transport chain and fatty acid oxidation (MacArthur et al., 2007, 2008). The shift of the fast fibers into more slow-twitch and oxidative fibers in the absence of the α-actinin-3 may be due to the fact that upregulated α-actinin-2 binds more calsarcin-2. Less calsarcin-2 bound to calcineurin enhances the calcineurin signaling and finally influences the slower rate of decline in calcium release from the sarcoplasmic reticulum following repeated muscle stimulation (Frey et al., 2008; Head et al., 2015; Kingsbury et al., 2000).

Despite the high sequence homology of the α-actinin-2 and α-actinin-3 they have different patterns of expression in skeletal muscle fiber, pointed at the specialized roles they play in the structure, signaling and metabolism of the muscle tissue. It has been observed that α-actinin-2 is able to compensate for, at least in part, the α-actinin-3 deficiency. However, the functional differences between products of *ACTN2* and *ACTN3* are probably due to their respective abilities to interact with different cellular proteins, particularly at the Z-line such as Z-band alternatively spliced PDZ motif containing protein (ZASP), myotilin, desmin, γ-filamin as well as vinculin and titin (Lee et al., 2016). Upregulation, different distribution and accumulation of these proteins may lead to muscle remodeling, suggesting that muscles of *Actn3* KO mice are more susceptible to contraction-induced damage of skeletal muscle (Lee et al., 2016; Seto et al., 2011). It has been observed that titin, a giant protein that spans the whole length of the sarcomere, from Z-discs to M lines, preferentially binds α-actinin-2 (Seto et al., 2011). Titin as an elastic molecular spring bears passive tension and is responsible for passive stiffness of the muscle

fibers (the details are provided in the next subsection). It was shown that in α-actinin-3 deficient fibers of *Actn3* KO mice, the higher binding affinity of titin to α-actinin-2 may change the concentration and organization of titin in the muscle fiber and results in increased fiber elasticity (Seto et al., 2011). In another animal experiment, it was proved that decreases in Young's modulus, which measures the stiffness of the muscle, were associated with the loss of the relative amount of titin (Toursel et al., 2002).

To assess the role of the α-actinin-3 gene R577X polymorphism in contractile properties of human muscle fibers, skinned single fibers from the quadriceps muscle of three men with spinal cord injury (SCI) were tested by Broos et al. (2012) regarding peak force, unloaded shortening velocity, the force-velocity relationship and passive tension (Broos et al., 2012). It was shown that absence of α-actinin-3 in XX homozygous individuals resulted in less stiff type IIa/IIx fibers. The heterozygote (RX) exhibited the highest fiber diameter and cross-sectional area (CSA) and the highest peak force. Another study by Broos et al. (2016) examined the effect of *ACTN3* R577X SNP on contractile and morphological properties of fast muscle fibers in 8 non-athletic young men. No differences were found in muscle fiber composition. However, the CSA of type IIa and IIx fibers was larger in RR homozygotes compared to XX individuals. Peak normalized force (P_0) was similar in both groups over all fiber types. A higher maximal unloading velocity (V_0) was observed in type IIa fibers of RR genotypes, but not in type I fibers. The visco-elasticity measured by Young's modulus and hysteresis was unaffected by a fiber type or genotype (Broos et al., 2012). To clarify whether the *ACTN3* R577X polymorphism influences passive stiffness of human muscle *in vivo*, and whether the R577X polymorphism is associated with the occurrence of hamstring strain injury, shear modulus of hamstring muscle (biceps femoris, semitendinosus, and semimembranosus) was assessed by Miyamoto et al. (2017). The muscle shear moduli of the semitendinosus and semimembranosus were significantly higher in R allele carriers than in XX homozygotes, whereas the shear modulus of the biceps femoris did not differ among the *ACTN3* R577X genotypes. The study indicated that presence of α-actinin-3 in type II muscle fibers was associated with increased passive muscle stiffness (mean stiffness of the hamstring being 15% lower in XX genotype carriers than in the R allele). However, the study did not show that altered mechanical property in α-actinin-3 deficient individuals might increase the risk of hamstring muscle strain injury (Miyamoto et al., 2017).

Two other studies performed by Kikuchi et al. (2017a, 2017b) evaluated the relationship between the *ACTN3* R577X genotype and functional characteristics of elbow flexors before and after isokinetic eccentric contractions (ECC), and examined the relationship between the *ACTN3* R577X polymorphism and trunk flexibility. In the first study, measurements of range of motion (ROM) from 90° flexion to 0° (full extension) were taken, among others, before, immediately after, and 1, 2, 3, and 5 days after ECC. RR individuals had lower flexibility than X allele carriers, as ROM in RR homozygotes at baseline was lower than that of X-allele carriers (Kikuchi et al., 2017a). The second experiment by Kikuchi et al. (2017b) investigated the association between *ACTN3* R577X polymorphisms and flexibility in nonathletic Japanese cohorts by assessing their sit-and-reach flexibility. The sit-and-reach in the RR genotype was significantly lower than in the RX and XX genotypes and it was concluded that RR individuals of *ACTN3* R577X in the general Japanese population had lower flexibility compared to the RX and XX genotypes (Kikuchi et al., 2017b).

It has been proposed that muscular flexibility plays a significant role in the development of injuries, sprains and overuse injuries; passive muscle stiffness affects joint flexibility and lower muscle flexibility has been considered an intrinsic risk factor for muscle strain injury (Garrett, 1996; Krivickas, 1997; Miyamoto et al., 2017; Witvrouw et al., 2003). Based on the above-mentioned data, it could be assumed that this effect would have been attributed to the possession of the R allele or RR genotype. However, it has been suggested that expression of α-actinin-3 may minimize muscle damage (Yang et al., 2003). Indeed, several studies showed that α-actinin-3 deficiency may increase the likelihood of muscle damage and enhance catabolic response after strenuous exercise (Deuster et al., 2013; Pimenta et al., 2011). For

example, XX individuals presented higher creatine kinase (CK) activity, α-actin concentration and higher levels of cortisol in comparison with RR individuals after acute eccentric training and people who possessed XX genotypes were also approximately three times more likely to develop exertional rhabdomyolysis compared to R allele carriers (Deuster et al., 2013; Pimenta et al., 2011). On the other hand, no such an association has been noted or even contrary results were obtained (Clarkson et al., 2005b; Venckunas et al., 2012). This discrepancy may, at least partly, be explained by the diverse mode of exercises used in the aforementioned studies. For instance, movements with purely eccentric actions have a different demand profile for the muscle–tendon unit compared to movements with repeated stretch–shortening cycles (Nicol et al., 2006). Therefore, it was proposed that α-actinin-3 deficiency might result in benefits to stretch–shortening cycle movements compared to R-allele carriers, whereas carrying the R allele would be beneficial in carrying out maximal eccentric contractions.

***TTN* gene**

Alterations in muscle fiber contractile characteristics as well as differences in fiber stiffness and visco-elasticity of the fiber were found in humans in many studies. The observed diversity probably corresponds to a fundamental reorganization of load-bearing proteins in the skeletal muscle. In this context the most obvious candidate is the aforementioned titin protein, encoded by the *TTN* gene. Titin (also known as a connectin) was discovered in late 70s (Maruyama, 1976) and described as the third most abundant muscle filament (after myosin and actin), responsible for elastic properties and scaffolding functions in the sarcomere as well as stretch-dependent tension development, intracellular mechanosensitivity and alterations in active contractile properties of muscle (Labeit et al., 1997). Many studies in humans and other mammals have proved that titin provides passive stiffness and modulates active contractile force of the muscles (Anderson and Granzier, 2012; Cazorla et al., 2001; Fukuda et al., 2003, 2005; Fukuda and Granzier, 2004; Granzier and Irving, 1995; Trombitás et al., 1995). In the heart muscle, titin plays a crucial role in sarcomeric assembly, stabilization and mechanosensing, and in cooperation with collagen, it is the main determinant of myocardial passive tension (LeWinter and Granzier, 2010).

Titin is the largest protein known to date (3-4 MDa) that extends from the Z-line (N-terminus of titin protein) to the M-line region (C-terminus of titin protein) of the sarcomere (Granzier and Labeit, 2005, 2006; Labeit and Kolmerer, 1995). The N-terminal part of the titin protein is embedded in the Z-disc by at least four binding sites for α-actinin, while the titin filaments from adjacent sarcomeres overlap within the Z-lines (Gregorio et al., 1998). In fact, titin amino-terminus acts as a part of mechanical stretch sensor machinery (Knöll et al., 2002) since the Z-disc, like other segments of the titin protein, is able to bind to the principal components of the sarcomere (Gautel and Djinović-Carugo, 2016; Linke, 2018; Seto et al., 2011). The above-mentioned titin-based passive force depends on the elastic segment forming the I-band region that spans between 0.8 and 1.5 MDa of the titin protein and functions as a molecular spring (Granzier and Irving, 1995; Linke, 2018; Wu et al., 2000). The central section of titin I-band is formed by a proximal and distal tandem Ig segments that are separated by the PEVK region (rich in proline, glutamic acid, valine, and lysine residues). The additional spring elements in this region are N2A (in skeletal and cardiac muscle) and N2B (in cardiac muscle only) segments (Bang et al., 2001; Helmes et al., 1999; Labeit and Kolmerer, 1995). When the PEVK domain was analyzed as a whole it displayed a spatially hierarchical arrangement of local elasticity: the N-terminal region was the most stiff, while the C-terminal region was the most flexible (Nagy et al., 2005). Towards the C-terminal part of titin protein the inextensible A-band is placed; this region contains Ig-like and fibronectin type III domains (Labeit and Kolmerer, 1995; Tskhovrebova and Trinick, 2004). The A-band of titin protein is structurally connected with the myosin filaments in the so-called I/A zone which is critical for the termination of the thick filament, while the C zone region of the titin A-band is engaged in anchoring myosin-binding protein C (MyBP-C) (Freiburg and Gautel, 1996), regulating actomyosin interaction (Muhle-Goll et al., 2001) as well as modification of the thick filament length (Tonino et al., 2017). The C-terminus of titin protein is

formed by the M-band that contains at the periphery the serine/ threonine kinase (TK) domain (Obermann et al., 1996) and is involved in numerous signaling pathways (Centner et al., 2001; Gotthardt et al., 2003; Peng et al., 2007; Witt et al., 2005b, 2005a). As it was said before, the titin filaments from opposite sarcomeres fully overlap in the Z-lines – a similar organization is found in the opposite terminus of the sarcomere: titin filaments from opposite half-sarcomeres fully overlap within the M-line region, that has scaffolding functions and probably conveys mechanosensitivity (Zacharchenko et al., 2015). Such overlapping titin ends bind to the other sarcomeric components, which constitute a contiguous system along myofibrils (Obermann et al., 1996).

As a very massive protein, titin is encoded by one of the largest genes – *TTN* located on the long arm of human chromosome 2 (locus: 2q31.2). Human *TTN* encompasses 365 exons of which 363 exons are coding exons (Bang et al., 2001). Alternative splicing of this gene results in multiple transcript variants, which causes the considerable variability in the I-band, the M-line as well as the Z-disc regions of titin and gives rise to isoforms with distinct spring compositions (Freiburg et al., 2000). Exon 49 coding for the N2B sequence is absent in titins specific for skeletal muscle, but is present in all cardiac titin isoforms. In the heart muscle two main titin expression variants are observed: (1) N2BA which has a longer PEVK segment and contains additional Ig domains, encoded by up to 313 exons, specifically: exon 49 (coding for N2B element) and exons 102–109 (coding for the N2A element) and (2) N2B, produced from 191 exons, specifically: exons 49/50 to exon 219 (Bang et al., 2001; Freiburg et al., 2000; Lahmers et al., 2004). In humans both cardiac isoforms differ in length of the I-band segments which causes the variability in myocardial stiffness (Cazorla et al., 2001; Freiburg et al., 2000; Wu et al., 2000) and are co-expressed with expression ratios N2BA:N2B of approximately 50:50 (range from 30:70 to 40:60) (Neagoe et al., 2002, 2003). Skeletal muscles are characterized by the expression of the N2A titin isoform, which is encoded by up to 312 exons – always without use of exon 49 (coding for the N2B element) (Bang et al., 2001; Freiburg et al., 2000). One common feature of all N2A titin isoforms in skeletal muscle is the longest PEVK segment when comparted to cardiac N2BA and N2B isoforms (Freiburg et al., 2000; Labeit and Kolmerer, 1995). Due to the processes of alternative splicing occurring during development of skeletal muscles many N2A titin isoforms of different length are generated. In different skeletal muscle types (Prado et al., 2005) as well as depending on the developmental stage (Ottenheijm et al., 2009), the N2A isoform diversity is large and a specific type of skeletal muscles appear to express a single titin isoform of determined length (Prado et al., 2005). Expressing a single titin isoform of appropriate size may be regarded as a modulation mechanism that enables the skeletal muscles to adjust the sarcomere's passive stiffness (Ottenheijm et al., 2009).

Molecular studies have highlighted that during the cardiac tissue development large N2BA and small N2B isoforms remain of the same size, but their expression ratio shifts towards the ratio characteristic for the adult myocardium (Krüger et al., 2008; Krüger and Linke, 2006; Lahmers et al., 2004; Warren et al., 2004). Quite a different situation is observed during the developmental processes of skeletal muscle: in neonatal skeletal muscle many titin isoforms can be expressed that are initially similar in length, but they gradually shift from larger to smaller in course of time (Ottenheijm et al., 2009). Such a developmental decrease in N2A isoform size mainly depends on alternative splicing that increases the proportion of E-rich motifs in the PEVK segment of the I-band region of titin protein. Taking into account that Ca2+ binding to E-rich motifs in the PEVK segment increases its bending rigidity, it could be speculated that a higher number of such motifs may increase Ca2+dependent titin-based passive stiffness in adult muscle fibers (Labeit et al., 2003). It is worth to notice that long titin springs are more compliant and elastic, while shorter isoforms with fewer exons spliced in are more rigid – it gives rise to substantial variability in titin's elastic properties as well as in titin-based passive tension in skeletal muscles (Linke et al., 1998). Extending PEVK fragments probably affects passive stiffness of titin by altering the PEVK's contour length and through changes in its hierarchical extensibility (Ottenheijm and Granzier, 2010). The described variability in the I-band region contributes to the differences in elasticity of different titin isoforms

and, therefore, to the differences in elasticity of different muscle types (Makarenko et al., 2004; Nagueh et al., 2004). All above mentioned changes that increase titin-based passive stiffness probably improve motor control during the muscle development (Ottenheijm and Granzier, 2010).

Not only are alternative splicing processes responsible for diversity of elasticity and stiffness of different titin isoforms, as many studies have revealed that titin protein undergoes posttranslational modifications which alter its elasticity and modify the titin-based passive stiffness of the muscles. Phosphorylation and oxidation alteration in the I-band part was frequently found in failing hearts which leads to the suggestion that these changes cause pathological myocardial stiffening in patients with systolic or diastolic heart failure (Beckendorf and Linke, 2015; Hamdani et al., 2017). Analyses performed on human and rat cardiac muscles have confirmed that phosphorylation of the N2B segment lowers titin stiffness (Hamdani et al., 2013; Krüger et al., 2009; Yamasaki et al., 2002), whereas phosphorylation of the constitutively expressed PEVK region increases it (Hidalgo et al., 2009). It is worth to note that in failing hearts the titin expression pattern is shifted toward a higher proportion of N2BA isoforms which are more elastic than stiffer N2B–titin springs (Makarenko et al., 2004; Nagueh et al., 2004; Neagoe et al., 2002). In consequence it lowers sarcomeric stiffness, which could be attributed to a compensatory mechanism preventing from the increased myocytes stiffness resulting from altered titin phosphorylation frequently observed in failing hearts (Linke, 2018).

Considering the key structural and functional roles played by titin protein in sarcomeres, it is not surprising that mutations in the *TTN* gene are investigated in the context of diseases of the skeletal and/or cardiac muscles. Muscular dystrophy (MD) is a group of diseases with genetic background resulting in progressive weakness and loss of skeletal muscle mass. Studies of patients suffering from MD revealed that mutations of *TTN* causing titin protein alterations were related to specific types of diseases from this group; such muscle disorders are known as titin myopathy or titinopathy and the severity as well as affected muscles are different depending on the position and type of the *TTN* mutations. The first described human titinopathy is tibial muscular dystrophy (TMD) (Udd et al., 1993) caused by insertion-deletion or missense mutations located in exon 363 encoding titin M-band (Hackman et al., 2008) as well as by mutations in exon 340 for A-band (Evilä et al., 2017) and other missense mutation in the A-band and frameshift mutations in *TTN* (Evilä et al., 2014). In next titinopathy – limb-girdle muscular dystrophy (LGMD) – a nonsense mutation in the last exon of *TTN* (Pénisson-Besnier et al., 2010) as well as a novel mutation designed as 107788T>C (W35930R) were described (Zheng et al., 2016). The truncating mutations in the C-terminal part of *TTN* were found in patients with Emery-Dreifuss muscular dystrophy (EDMD) (De Cid et al., 2015), while 274375T>C (C30071R) and G30150D mutations in the A-band encoding part of the *TTN* gene were identified in patients suffering from hereditary myopathy with early respiratory failure (HMERF) (Pfeffer et al., 2012; Toro et al., 2013). The whole-exome sequencing revealed that mutations located in M-band encoding the *TTN* gene region were present in patients with central core myopathy (CCM) (Chauveau et al., 2014), while titin truncating mutations and nonsense mutations in the I-band region were characteristic for patients with centronuclear myopathy (Ceyhan-Birsoy et al., 2013; Fattori et al., 2015); *TTN* deletion mutations were described in the M-band coding region in patients with the so-called Salih myopathy (Carmignac et al., 2007).

To sum up, all aforementioned studies have shown that different *TTN* gene mutations (both the insertion/deletion changes causing creation of truncated titin isoforms unable to span between the M-line and the Z-disk as well as missense mutations that give rise to normal-sized titin isoforms poorly attached to the Z-disk) may result in originating titin with altered elastic properties and, in consequence, sarcomeres with impaired contractile characteristics. Taken together, studies on *TTN* gene variants have provided evidence of titin-related structure-function relationships in the heart and skeletal muscle.

Summary

Our current knowledge of muscle characteristics indicates that muscles are complex

organs, both in the context of structural and functional properties, with all components working together to produce complex responses to changes in activation and length. Although it is known that skeletal muscle is extremely plastic and can adapt its elasticity and stiffness under dynamic conditions to generate appropriate amount of power, there is still much unknown about the genetic background of the development and functional regulation of these muscle's properties. Investigation of the interactions between the presence of specific genetic variants and characteristics of muscular systems will provide information whether and how muscles adapt to their function and environmental changes at the molecular level. We assume that better understanding of the genetic background of sport-related traits, such as muscle stiffness, muscle elasticity and explosive strength, will allow the idea of personalized training based on the athlete's genetic profile to be used in practice. Therefore, it is likely that genetic tests based on polymorphic sites of certain genes will become a supplementary tool that helps coaches improve as well as adapt more precise training methods what in consequence allow athletes to achieve better results in response to training.

In this review, we attempted to identify genetic factors that may have impact on such muscle properties as elasticity, stiffness and strength.

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