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Gene polymorphisms and fibre type composition of human skeletal muscle

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Abstract

The ability to perform aerobic or anaerobic exercise widely varies among individuals, partially depending on their muscle fibre composition. Variability in the proportion of skeletal muscle fibre types may also explain marked differences in aspects of certain chronic disease states, including obesity, insulin resistance and hypertension. In untrained individuals, the proportion of slow-twitch (type I) fibres in the vastus lateralis muscle is typically around 50% (range 5-90%) and it is unusual for them to undergo conversion to fast-twitch fibres. It has been suggested that the genetic component for the observed variability in the proportion of type I fibres in human muscles is of the order of 40 to 50%, indicating that muscle fibre type composition is determined both by genotype and environment. This article briefly reviews current progress in the understanding of genetic determinism of fibre type proportion in human skeletal muscle. Several polymorphisms of genes involved in calcineurin/NFAT pathway, mitochondrial biogenesis, glucose and lipid metabolism, cytoskeletal function, hypoxia/angiogenesis and circulatory homeostasis have been associated with fibre type composition. As muscle is a major contributor to metabolism and physical strength, and can readily adapt, it is not surprising that many of these gene variants have been associated with physical performance and athlete status, as well as metabolic and cardiovascular diseases. Genetic variants associated with fibre type proportions have important implications for our understanding of muscle function in both health and disease.

Keywords muscle function; sport physiology; metabolism; body composition; fibre type proportion

Introduction

Human skeletal muscle is a heterogeneous tissue composed of two main fibre types, which were classified as type I and II with subgroups IIA and IIB on the basis of myosin ATPase histochemical staining (Brooke and Kaiser 1970). Immunohistochemical staining with antibodies specific for different myosin heavy chain (MyHC) isoforms and in situ hybridization analyses aimed to detect MyHC transcripts showed that human IIB fibres correspond in fact to IIX fibres, containing a MyHC IIX similar to that present in type IIX fibres of mouse and rat muscle, whereas IIB MyHC is not expressed in human skeletal muscle (Smerdu et al. 1994). These fibre types differ in maximal velocity of shortening, type I fibres showing the slowest contraction properties and type IIX the fastest, as determined by studies on single human fibres with defined MyHC composition (Larsson and Moss 1993; Bottinelli and Reggiani 2000). When combined with a larger mean cross-sectional area (at least in men; Staron et al. 2000), the greater maximal velocity of shortening (Larsson and Moss 1993; Bottinelli and Reggiani 2000) of type II and especially type IIX fibres (both illustrated in Fig. 1) means that they can produce substantially greater maximal power compared with type I fibres. Indeed, power (rather than strength or size *per se*) is frequently a major factor in determining sporting success at one extreme of the human performance continuum and functional ability in daily tasks at the other extreme (Wilson et al. 1993). This is because few sporting or daily tasks involve muscle contractions at the extremes of the force-velocity relationship where either the speed of movement is zero (i.e. a maximal isometric contraction, where power produced is zero) or the force produced is zero (i.e. a maximal velocity contraction, where power production is again also zero). Enzyme histochemical studies showed that type I fibres are rich in oxidative enzymes but relatively poor in glycolytic enzymes, whereas type IIX fibres have high glycolytic enzyme activity and low levels of oxidative enzymes, and type IIA fibres have intermediate properties (Essen et al. 1975). Similar conclusions were reached with microchemical analyses of single fibres defined with myosin ATPase (Hintz et al. 1984). Known determinants of fibre type in human skeletal muscle are innervation, intensity of different types of training, spaceflight and unloading, thyroid hormone level and disease states (reviewed in Gundersen 1998; Baldwin and Haddad 2001). In addition, in terms of epigenetics, muscle fibre types I, IIA and IIX, and their hybrids (I/IIA, IIA/IIX), contain essentially the same DNA, but possess different gene expression profiling (epigenomes) which also appear to influence their properties (Baar 2010). Based on their myosin and metabolic profile, the data shows that type I fibres have high resistance to fatigue and are thus suited for endurance performance, IIA fibres better suited for medium-term anaerobic exercise, and type IIX fibres adapted for short bursts of high speed and power (reviewed in Gollnick and Matoba 1984; Andersen et al. 2000).

Vastus lateralis is a major muscle that is involved in propulsive and ambulatory activities. It is the most commonly sampled muscle in the literature. The proportion of type I fibres in the vastus lateralis, is typically around 50% but there are wide variations (range ~5-90%) (Simoneau and Bouchard 1989; Klitgaard et al. 1990; Andersen et al. 2000, Andersen and Aagaard 2000; Staron et al. 2000). This phenomenon may, in part, explain the observations that individuals have different capacities to perform aerobic or anaerobic exercise (Saltin and Gollnick 1983). Endurance-oriented athletes are reported to have a remarkably high proportion of type I fibres in their trained muscle groups (Ricoy et al. 1998; Zawadowska et al. 2004), whereas muscles of sprinters and weightlifters predominantly consist of IIA/IIX fibres (Andersen et al. 1994). It is well known that fibre proportions vary between different muscles in humans (and other species) (Johnson et al. 1973). A recent paper by Vikne et al. (2011) has shown that those expressing a relatively large proportion of type I fibres in one muscle will also express a relatively large proportion of these fibres in other muscles. Although more evidence is needed, sampling just one muscle in all subjects may still be a valuable indicator of overall relative fibre proportion. Nevertheless, it should also be noted that there exist potential variations in fibre type distribution and size from superficial to deep and proximal to distal, within the same muscle group (Elder et al. 1982; Lexell and Taylor 1989).

Since muscle mass is highly metabolically active (Elia 1992), accounts for ~23% of total resting energy expenditure (Gallagher et al. 1998) and makes up a high proportion of total body mass (~30-40%; Janssen et al. 2000), it strongly influences overall metabolism. Oxidative muscle fibres utilize primarily fatty acids and frequent use of these muscle fibres leads to the reduction of fat mass and improvement of insulin sensitivity (Kriketos et al. 1996; Toft et al. 1998). Variability in the proportion of skeletal muscle fibres have been investigated and been found to contribute to susceptibility and aspects of chronic disease states such as obesity, type 2 diabetes, insulin resistance and hypertension (Bassett 1994). Wade et al. (1990) have shown a negative correlation between the proportion of type I muscle fibres and body fat percentage. Furthermore, 40% of the variability in body fat percentage could be explained by muscle fibre type composition (Wade et al. 1990). Accordingly, low percentage of type I muscle fibres was shown to be a risk factor for the development of obesity and insulin resistance (Lillioja et al. 1987; Sun et al. 2002; Tanner et al. 2002; Gerrits et al. 2010). Additionally, Frisk-Holmberg et al. (1983) reported that hypertensive subjects had a tendency (though not statistically significant in this case) to possess a higher proportion of fast-twitch fibres (, which may be partly explained by the positive correlation between fat mass and hypertension. Furthermore, Hernandez et al. (2001) reported that the percentage of type IIB fibres was related to diastolic blood pressure in normotensive men and to mean blood pressure in hypertensive subjects.

It is certainly true that plasticity in fibre type composition, in humans, has been demonstrated on numerous occasions. Exercise training of all kinds (resistance, aerobic or mix training) tends to induce a fast-to-slow transition in fibre type, especially a shift from type IIX to type IIA (Kraemer et al. 1995; Pette and Staron 1997; Pette 1998; Canepari et al. 2005). Detraining or denervation, on the other hand, tends to induce a slow-to-fast transition (Pette and Staron 1997; Biering-Sorensen et al. 2009). Interestingly, careful manipulation of training loads that includes a final period of detraining appears able to induce an 'overshoot' in the proportion of type IIX MyHC (Andersen and Aagaard 2000). Nevertheless, on the basis of comparative analyses of fibre type composition in monozygotic and dizygotic twins and normal brothers, In arguably the most well-conducted study of its kind on the topic, Simoneau and Bouchard (1995) concluded that the genetic component for the proportion of type I fibres in human muscles is of the order of 40 to 50%, indicating that muscle fibre type composition is determined both by genotype and environment (muscle sampling and technical error component accounted for 15% of the total variance in the proportion of type I muscle fibres). The genetic variance is that portion of inter-individual phenotypic differences associated with sequence variations in DNA sequence. Genetic variance therefore includes the effects of single genes, gene-environment interaction, as well as gene-gene interaction. Not included within genetic variance are therefore all environmental factors including dietary intake, physical activity levels, intrauterine local environmental factors that effectively pre-date voluntary nutritional patterns and physical activity, and other lifestyle components that may be influenced by the social and physical environment (Simoneau and Bouchard 1995; Matsakas and Patel 2009). Such environmental factors modulate muscle phenotype via epigenetic mechanisms (methylation/demethylation, acetylation/deacetylation, RNA-mediated processes, regulation of translation) (Baar 2010; Gibney and Nolan 2010), activation of transcription factors (such as myf5, myoD, MRF4, myogenin, NFATs, PPARδ) and activation of transcriptional coactivators (calcineurin, PGC-1α, PGC-1β) (Fluck and Hoppeler 2003; Wang et al. 2004; Arany et al. 2007). Whether epigenetic changes (i.e. stable and heritable changes in gene expression) in skeletal muscle can be passed to the next generation, and consequently form part of the heritable influence on muscle fibre composition, has yet to be determined.

The main goal of this article is to review current progress in the understanding of genetic determinism of fibre type proportion in human skeletal muscle.

Gene polymorphisms associated with interindividual differences in muscle fibre composition

To date, there have been reports that 14 gene polymorphisms individually or in combination are associated with muscle fibre composition (Table 1). These reports are under the six headings below.

ACE

Circulating angiotensin I converting enzyme (ACE) exerts a tonic regulatory function in circulatory homeostasis, through the synthesis of vasoconstrictor angiotensin II, which also drives aldosterone synthesis, and the degradation of vasodilator kinins. It was shown that *ACE* deletion (D) allele (of the Alu I/D polymorphism) is associated with high serum and tissue ACE activity, hypertension, type 2 diabetes, obesity, coronary heart disease and myocardial infarction (Kennon et al. 1999; Rieder and Nickerson 2000; Strazzullo et al. 2003). An excess of the *ACE* insertion (I) allele was found in endurance-oriented athletes compared to controls in several studies (reviewed in Ahmetov and Rogozkin 2009), indicating that *ACE* I allele is favourable for aerobic performance. In a hypertensive and insulin-resistant animal model (fructose-fed rats), ACE inhibitor temocapril was shown to produce a recovery of the composition ratio of type I fibre of soleus muscle to the same as control and improved insulin sensitivity (Higashiura et al. 2000). In 2003, Zhang and colleagues tested the hypothesis that the *ACE* gene polymorphism may influence muscle characteristics in humans which could in part explain the association of gene variation with endurance performance. Indeed, they revealed that the greater the I allele frequencies, the higher percentage of type I skeletal muscle fibres (determined by staining for myosin ATPase activity) and the greater the D allele frequency, the higher percentage of type II fibres in *m. vastus lateralis* of Japanese healthy subjects (31 males, 10 females, age 24 ± 3 years) (Zhang et al. 2003). They then examined the histochemical characteristics of soleus muscle in the *Ace* knockout mice. However, in both male and female mice, the composition of fibre types (type I and IIA) did not differ significantly between $Ace^{+/+}$ and $Ace^{+/-}$ mice (Zhang et al. 2005). One paper which examined muscle fibre type and *ACE* polymorphism was not included in this review (Akhmetov et al. 2006). The genotyping method detailed in this paper (the use of only 2 primers for amplification) could have resulted in mistyping of the ID as the DD genotype (Shanmugam et al. 1993).

ACTN3

The α-actinins constitute the predominant protein component of the sarcomeric Z line in skeletal muscle fibres, where they form a lattice structure that anchors together actin containing thin filaments and stabilizes the muscle contractile apparatus. Expression of the α-actininin-3 (ACTN3) is limited to fast muscle fibres responsible for generating force at high velocity. A common genetic variation in the *ACTN3* gene that results in the replacement of an arginine with a stop codon at amino acid 577 (C-to-T transition in exon 16; rs1815739; R577X) had been identified. The 577X allele contains a sequence change that completely prevents the production of functional α-actinin-3 protein (North et al. 1999).

Several case-control studies reported that *ACTN3* RR genotype is over-represented or *ACTN3* XX genotype is under-represented in strength/sprint athletes in comparison with controls (reviewed in Yang et al. 2009). Additional meta-analysis using nine studies confirmed this kind of association (Alfred et al. 2011). Vincent et al. (2007) have shown that the cross-sectional area and number of type IIX fibres of *m. vastus lateralis* (determined by immunohistochemistry) was greater in the RR than the XX genotype group of young healthy men (n = 44; aged 18-29). We have recently observed a similar relationship between *ACTN3* R577X polymorphism and muscle fibre composition in 94 subjects (of which 60 were physically active healthy men and 34 were speed skaters), indicating that *ACTN3* XX genotype carriers exhibit a higher proportion of slow-twitch fibres (determined by immunohistochemistry). *ACTN3* genotype explained 4.6% of the variation in muscle fibre composition of m. vastus lateralis (Ahmetov et al. 2011) – a notably high percentage for just one single polymorphism. Furthermore, Norman et al. (2009) found a slightly (but not significantly) higher proportion of type IIA muscle fibres (determined using the myofibrillar ATPase histochemical stain) in subjects of RR genotype (39 ± 14%) in comparison with RX (36 ± 6%) and XX (31 ± 15%) genotypes of 37 young men but not in young women (n = 26). MacArthur et al. (2008) have shown that muscle from *Actn3* knockout mice displays reduced force generation and fast fibre diameter, increased activity of aerobic enzymes, and enhanced recovery from fatigue, suggesting a shift in the properties of fast fibres towards those characteristic of slow fibres. However, this observation was not supported by the evidence of direct change of muscle fibre composition. Interestingly, even though fibre properties were significantly altered, there was no change in MyHC proportions. If their fast-twitch fibres share properties of the slow-twitch fibres, alterations in fibre type composition in the mouse model may only be seen with training. One possible explanation for the reported relationship between α-actininin-3 deficiency (*ACTN3* XX genotype) and slow-twitch muscle fibre phenotype could be evidence that α-actinins interact with signaling proteins, such as calcineurin (reviewed in MacArthur and North 2004). Importantly, calcineurin is known to play a key role in the determination of muscle fibre type and muscle hypertrophy (Olson and Williams 2000).

HIF1A

Glycolysis is the central source of anaerobic energy in humans, and this metabolic pathway is regulated under low-oxygen conditions by the transcription factor hypoxia-inducible factor 1α (HIF1α; encoded by *HIF1A*). HIF1α controls the expression of several genes implicated in various cellular functions including glucose metabolism and angiogenesis. HIF-1α mRNA and protein levels were found to be constitutively higher in the more glycolytic muscles compared with the more oxidative muscles (Pisani and Dechesne, 2005). A lower proportion of type IIA fibres in the soleus muscles of

HIF-1a knockout mice was detected as well as a metabolic shift away from glycolysis toward oxidation, and as a consequence, improved endurance capacity (Mason et al. 2004). Lunde et al. (2011) have shown that when HIF-1α was overexpressed for 14 days after somatic gene transfer in adult rats, a slow-to-fast transformation was observed. In humans, a missense polymorphism in the *HIF1A* gene, Pro582Ser, is present in exon 12 (rs11549465 C/T). The rare T allele is predicted to result in a proline to serine change in the amino acid sequence of the protein. This substitution increases HIF1α protein stability and transcriptional activity (Tanimoto et al. 2003), and therefore, may improve glucose metabolism and lower the risk of type 2 diabetes (Nagy et al. 2009). Prior et al. (2003) have shown that *HIF1A* Pro/Pro homozygotes showed preservation of the ability to increase VO_{2max} through aerobic exercise training at each age (55, 60 and 65 yr) level evaluated. Contrary to this, subjects carrying the Ser allele were able to increase VO_{2max} to a similar extent as Pro/Pro homozygotes at 55 yr of age, but showed significantly less increase in VO_{2max} to aerobic exercise training than Pro/Pro homozygotes at 60 and 65 yr of age. The *HIF1A* 582Ser allele was shown to be associated with an increased proportion of fast-twitch muscle fibres (Pro/Ser – 46.2 (13.8)%, Pro/Pro – 31.4 (8.2)%) in *m. vastus lateralis* (determined by immunohistochemistry) of 21 Russian all-round speed skaters (14 males and 7 females; age 20.5 ± 0.5 years) and the frequency of this allele was significantly higher in weightlifters in comparison with controls (Ahmetov et al. 2008). On the other hand, Döring et al. (2010) by studying 316 Caucasian male elite endurance athletes from the Genathlete cohort and 304 Caucasian male sedentary controls have found that the opposing Pro582 allele is associated with endurance athlete status, which would be consistent with the data from Ahmetov et al. (2008) and Prior et al. (2003).

PPARA

Peroxisome proliferator-activated receptor α (PPARα) is a transcription factor that regulates lipid, glucose and energy homeostasis. Endurance training increases the use of non-plasma fatty acids and may enhance skeletal muscle oxidative capacity by PPARα regulation of gene expression. The level of expression of PPARα is higher in type I than in type II muscle fibres (Russel et al. 2003). Exercise-induced left ventricular (LV) growth in healthy young men was strongly associated with the intron 7 G/C (rs4253778) polymorphism of the *PPARA* gene (Jamshidi et al. 2002). Individuals homozygous for the C allele had a 3-fold greater and heterozygotes had a 2-fold greater increase in LV mass than G allele homozygotes, leading to the hypothesis that the hypertrophic effect of the rare intron 7 C allele is due to influences on cardiac substrate utilization. Furthermore, the *PPARA* rs4253778 C allele was shown to be associated with type 2 diabetes and atherosclerosis (Flavell et al. 2002, 2005). Recently, it was demonstrated that the frequency of the *PPARA* rs4253778 GG genotype was significantly higher

in Russian endurance-oriented athletes (Ahmetov et al. 2006), elite Israeli endurance athletes (Eynon et al. 2010) and elite Polish rowers compared to controls and/or sprinters (Maciejewska et al. 2010). In accordance with the hypothesis that the observations of allele frequency differences amongst athletes are mediated via fibre type, the mean percentage of type I muscle fibre (determined by immunohistochemistry) was higher in GG homozygotes (55.5 ± 2.0%) than in CC genotype subjects (38.5 ± 2.3%) in 40 physically active healthy men (Ahmetov et al. 2006).

VEGFR2

Vascular endothelial growth factor (VEGF) is a major growth factor for endothelial cells and VEGF receptor 2 (VEGFR2; also known as kinase insert domain receptor, KDR) is essential to induce the full spectrum of VEGF angiogenic responses to aerobic training. *VEGFR2* mRNA expression is increased by acute systemic exercise (Gavin et al. 2004, 2007; Gustafsson et al. 2007). One of the potential functional polymorphisms of the *VEGFR2* gene is the rs1870377 T/A variant, which determines a histidine (His) to glutamine (Gln) substitution of the receptor. Studies have reported that the His472Gln polymorphism influences the efficiency of VEGF binding to VEGFR2 (Wang et al. 2007; Zhang et al. 2007) and is associated with clinical phenotypes such as coronary heart disease, stroke, cancer and exceptional longevity (Ellis et al. 2007; Försti et al. 2007; Wang et al. 2007; Zhang et al. 2007; Sebastiani et al. 2008). We have recently found that the frequency of the *VEGFR2* 472Gln allele was significantly higher in endurance-oriented Russian athletes compared to controls. The 472Gln allele was also shown to be significantly related to a higher proportion of type I fibres of *m. vastus lateralis* (determined by immunohistochemistry) in both athletes (all-round speed skaters, n = 23; age 20.4 ± 0.5 years) and physically-active men (n = 45; age 23.5 ± 0.4 years) (Ahmetov et al. 2009a).

Combined impact of gene variants on muscle fibre composition

Human skeletal muscle phenotypes are classical quantitative traits influenced by many gene variants and environmental factors. It is important to note that each DNA locus can typically explain only a small proportion of the phenotypic variance. Therefore, large sample sizes are needed to detect associations with single polymorphisms and various combinatorial approaches should be used where the phenotypic variance associated with several genetic variants can be assessed simultaneously. We have recently quantified the association between a combination of genotypes and fibre composition of *m. vastus lateralis* (determined by immunohistochemistry) in 45 healthy men (age 23.5 ± 0.4 years) (Ahmetov et al. 2009b). For this analysis, we used 10 polymorphisms of genes involved in the calcineurin/NFAT pathway (*PPP3R1* promoter 5I/5D, *NFATC4* Gly160Ala), mitochondrial biogenesis (*PPARGC1A* Gly482Ser, *PPARGC1B* Ala203Pro, *TFAM* Ser12Thr), glucose and lipid metabolism

(*PPARA* rs4253778 G/C, *PPARD* rs2016520 T/C), hypoxia/angiogenesis (*VEGFA* rs2010963 G/C) and thermogenesis (*UCP2* Ala55Val, *UCP3* rs1800849 C/T). Of these 10 genes, the *NFATC4*, *PPARA*, *PPARD*, *PPARGC1A*, *PPARGC1B*, *PPP3R1* and *TFAM* genes code for transcription factors and coactivators, while *UCP2*, *UCP3* and *VEGFA* represent their target genes.

Importantly, studies using transgenic or knockout rodent models have shown the significance of calcineurin, NFATc4, PPARδ, PGC-1α and PGC-1β in the regulation of muscle fibre composition (Chin et al. 1998; Naya et al. 2001; Serrano et al. 2001; Lin et al. 2002; Luquet et al. 2003; McCullagh et al. 2004; Wang et al. 2004; Schuler et al. 2006; Arany et al. 2007; Calabria et al. 2009). More specifically, activation of the expression of PGC-1α, PGC-1β, PPARδ and calcineurin genes leads to the increase of the proportion of oxidative muscle fibres (Naya et al. 2001; Chin et al. 2002; Lin et al. 2002; Luquet et al. 2003; Wang et al. 2004; Arany et al. 2007), while knockouting of PPARδ gene or inhibition of activity of calcineurin causes a slow to fast fibre transition (Serrano et al. 2001; Chin et al. 2002; McCullagh et al. 2004; Schuler et al. 2006). Furthermore, Calabria et al. (2009) have shown inhibition of fast-glycolytic MyHC-2B by siRNAs for NFATc4.

In accordance with the hypothesis, the number of 'endurance' alleles (i.e. the alleles individually associated with endurance athlete status or related phenotypes; *PPP3R1* 5I, *NFATC4* Gly160, *PPARGC1A* Gly482, *PPARGC1B* 203Pro, *TFAM* 12Thr, *PPARA* rs4253778 G, *PPARD* rs2016520 C, *VEGFA* rs2010963 C, *UCP2* 55Val, *UCP3* rs1800849 T) was positively correlated ($r = 0.50$; $P = 4.0 \times 10^{-4}$) with the proportion of slow-twitch fibres. Specifically, for men with high numbers (≥ 9) of 'endurance' alleles ($n = 26$) compared to those with low numbers (≤ 8) of 'endurance' alleles ($n = 19$), there was a greater number of slow-twitch fibres in the *m. vastus lateralis* (56.1 ± 1.8% vs. 43.8 ± 2.2%; $P = 1.0 \times 10^{-4}$; these results remained statistically significant after correction for multiple testing) and a higher proportion of area occupied by those fibres (50.0% vs. 41.8%; $P = 0.033$) (Ahmetov et al. 2009b).

It should also be noted that the *PPARGC1A* Gly482, *PPARGC1B* 203Pro, *PPARD* rs2016520 C, *UCP2* 55Val, *UCP3* rs1800849 T alleles are associated with low risk of obesity (Halsall et al. 2001; Andersen et al. 2005; Aberle et al. 2006; Liu et al. 2005b; Ridderstråle et al. 2006), type 2 diabetes (Fanelli et al. 2005; Barroso et al. 2006) and hypertension (Vimaleswaran et al. 2008). Collectively, these findings suggest that the association of DNA polymorphisms with elite athlete status and several diseases (extended phenotypes) can be explained, in part, by their relationship with muscle fibre composition (intermediate phenotype).

Possible mechanisms underlying the association between gene polymorphisms and muscle fibre composition

There is a tremendous degree of plasticity in MyHC gene expression that can be induced in striated muscle, depending on the environmental conditions imposed on a given muscle (reviewed in Baldwin and Haddad 2001). For example, in rodents, a high fat diet has been shown to induce an oxidative profile in skeletal muscle; on the other hand, food restriction has been shown to decrease the myofibre cross sectional area (reviewed in Matsakas and Patel 2009). However, the extent of gene expression may be restricted in any given fibre. One possible explanation for the putative limited range of adaptation may lie in the existence of discrete populations of myoblasts, each giving rise to a specific subset of myonuclei with distinct patterns of gene expression (Stockdale 1992). The population of primary myotubes that continue to express only the slow isoform tends to be localized to the deeper portions of larger muscles. Because these are the areas that, in the adult, are the site of type I fibres, this led to the suggestion that primary myotubes eventually develop into adult slow fibres, whereas secondary myotubes develop into fast fibres (Condon et al. 1990). Given the fact that most muscle fibres will contain myonuclei of both origins, the MyHC expression of any fibre will reflect the proportions of primary and secondary myonuclei (reviewed in Parry 2001). If epigenetic imprinting through DNA methylation, acetylation/deacetylation and other events play a significant role in the determination of cell fate during development (Baar 2010), then one might speculate that polymorphisms of genes involved in such types of epigenetic processes could explain why some individuals can exhibit a high or a low percentage of different fibre types. This hypothesis should be tested in future studies.

Significant progress has been made during the last several years in the identification of the signaling pathways that control muscle fibre types. The function of specific genes has been defined by gain- and loss-of function approaches using transgenic and knockout mouse models. These genes are involved in calcineurin/NFAT, PGC-1/PPARδ, Ca/CaMK/HDAC (calcium/calmodulin-dependent protein kinase and histone deacetylases), thyroid hormone and other pathways (Liu et al. 2005a; Arany 2008; Simonides and van Hardeveld 2008; reviewed in Schiaffino 2010). It can be suggested that DNA polymorphisms which influence gene expression of these signaling pathways predispose the muscle precursor cells of a given individual to be predominantly fast or slow. Consequently, gene variations could be considered as molecular determinants maintaining the expression of the slow or fast MyHC of adult skeletal muscle. The functional properties of the majority of the gene polymorphisms described in the current review (*ACE* I/D, *ACTN3* R577X, *HIF1A* Pro582Ser, *PPARD* rs2016520, *PPARGC1A* rs8192678, *PPARGC1B* rs7732671, *PPP3R1* promoter 5I/5D, *UCP2* rs660339, *UCP3* rs1800849 and *VEGFA* rs2010963) have been proposed (Danser et al. 1995; North et al. 1999; Schrauwen et al. 1999; Watson et al. 2000; Buemann et al. 2001; Skogsberg et al. 2003; Tanimoto et al. 2003; Ling et al. 2004; Tang et al. 2005; Ling et al. 2007). These gene variations are associated

with differences in gene expression or protein structure and therefore, could partly explain interindividual differences in the proportion of muscle fibres. Factors associated with muscle fibre composition are shown in Table 2.

Conclusion

The current review provides evidence that polymorphisms of genes involved in calcineurin/NFAT pathway, mitochondrial biogenesis, glucose and lipid metabolism, cytoskeletal function, hypoxia/angiogenesis and circulatory homeostasis may explain, in part, the observed interindividual variability in muscle fibre composition. Interestingly, most of these gene variants have also been associated with either physical performance, athlete status or different metabolic and cardiovascular diseases, or indeed several of these, indicating that these phenotypes may share some common molecular mechanism of development. However, it should be emphasized that most of the association studies have not yet been replicated in independent cohorts. Such types of studies generally have low sample sizes (less than 100 subjects) because of the invasiveness of muscle biopsies, and large samples are difficult to achieve. Furthermore, when examining potential polymorphism for associations there are unknown linkage with other variants, environmental interactions, or epistatic (gene-gene interaction) effects. Differences in methods for the determination of muscle fibres may also influence the results of association studies. Future research that embraces the advancing technology available in genomics, such as gene chips with wide genomic coverage or next generation sequencing, will be needed to determine a more comprehensive list of the important polymorphisms involved in the regulation of muscle fibre composition – genes encoding for transcription factors such as myf5, myoD, MRF4 and myogenin could be targets for deep sequencing in this context. Additionally, improved immunohistochemistry methods and automated methods for fibre proportion analysis could help provide further knowledge on current suggested gene variants and other potential polymorphisms. Such studies are important for our understanding of muscle function in both health and disease.

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Table 1. Gene polymorphisms individually or in combination associated with muscle fibre composition.

Gene	Location	Polymorphism	Allele associated with increased proportion of type I muscle fibres	Sample size	References
ACE	17q23.3	Alu I/D	I	41	Zhang et al. 2003
ACTN3	11q13.1	R577X (rs1815739 C/T)	577X	44 and 94	Vincent et al. 2007; Ahmetov et al. 2011
HIF1A	14q21-q24	Pro582Ser (rs11549465 C/T)	Pro582	21	Ahmetov et al. 2008
NFATC4	14q11.2	Gly160Ala (rs2229309 G/C)	Gly160	45	Ahmetov et al. 2009b
PPARA	22q13.31	rs4253778 G/C	rs4253778 G	40	Ahmetov et al. 2006
PPARD	6p21.2-p21.1	rs2016520 T/C	rs2016520 C	45	Ahmetov et al. 2009b
PPARGC1A	4p15.1	Gly482Ser (rs8192678 G/A)	Gly482	45	Ahmetov et al. 2009b
PPARGC1B	5q33.1	Ala203Pro (rs7732671 G/C)	203Pro	45	Ahmetov et al. 2009b
PPP3R1	2p15	Promoter 5I/5D	5I	45	Ahmetov et al. 2009b
TFAM	10q21	Ser12Thr (rs1937 G/C)	12Thr	45	Ahmetov et al. 2009b
UCP2	11q13	Ala55Val (rs660339 C/T)	55Val	45	Ahmetov et al. 2009b
UCP3	11q13	rs1800849 C/T	rs1800849 T	45	Ahmetov et al. 2009b
VEGFA	6p12	rs2010963 G/C	rs2010963 C	45	Ahmetov et al. 2009b
VEGFR2	4q11-q12	rs1870377 T/A (His472Gln)	472Gln	68	Ahmetov et al. 2009a

Table 2. Factors associated with muscle fibre composition.

Factors	Factors which induce increased proportion of type I muscle fibres	Factors which induce increased proportion of type II muscle fibres
Environmental (intrinsic or extrinsic)	Tonic activity; reduced thyroid hormone level (hypothyroidism); high-intensity endurance training	Phasic pattern of activity; increased thyroid hormone level (hyperthyroidism); resistance training; spaceflight and unloading; spinal cord injuries
Genetic	Gene variants associated with high percentage of type I muscle fibres (e.g. *ACE* I, *ACTN3* 577X, *HIF1A* Pro582, *PPARA* rs4253778 G, *VEGFR2* 472Gln)	Gene variants associated with high percentage of type II muscle fibres (e.g. *ACE* D, *ACTN3* R577, *HIF1A* 582Ser, *PPARA* rs4253778 C, *VEGFR2* His472)

Note that environmental factors modulate muscle phenotype via epigenetic mechanisms and regulation of transcription factors. Consequently, the magnitude of change in muscle phenotype in response to environmental factors may partly depend on polymorphisms of genes involved in epigenetic processes (gene-environment interactions) and genes of transcription factors.

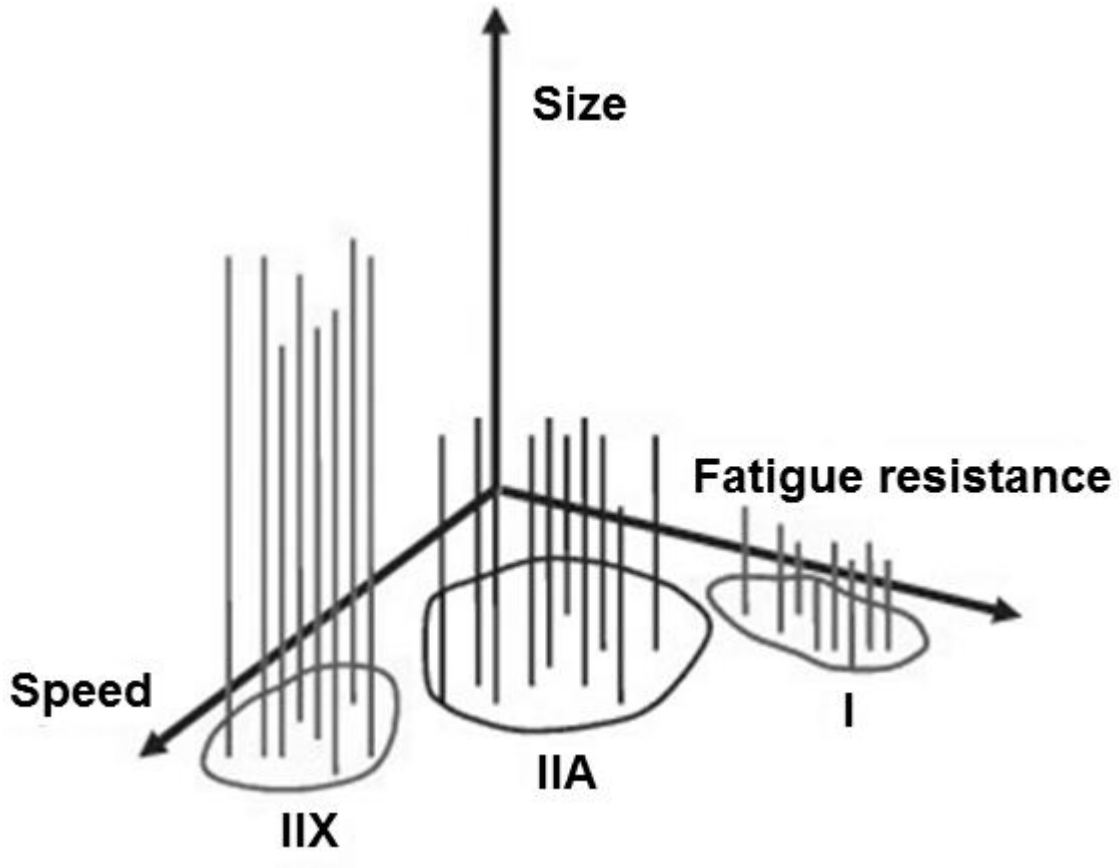

Figure 1. Contractile properties of motor units: the size, speed and fatigue resistance of different types of motor unit, as defined by histochemical properties. Type IIX motor units tend to be larger and contract more quickly but have lower fatigue resistance, while type I motor units tend to be smaller and contract more slowly but have greater fatigue resistance. Reprinted, by permission, from Jones D, Round J and de Haan A, 2004. *Skeletal Muscle: From Molecules to Movement* (Churchill Livingstone, Philadelphia).