Why is α-Actinin-3 Deficiency So Common in the General Population? The Evolution of Athletic Performance

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'We can now explain how this common genetic variation influences athletic performance as well as why it has become so common in the general population. There is a fascinating link between factors that influence survival in ancient humans and the factors that contribute to athletic abilities in modern man.'

The human *ACTN3* gene encodes the protein α-actinin-3, a component of the contractile apparatus in fast skeletal muscle fibers. In 1999, we identified a common polymorphism in *ACTN3* (R577X) that results in absence of α-actinin-3 in more than one billion people worldwide, despite the *ACTN3* gene being highly conserved during human evolution. In 2003, we demonstrated that *ACTN3* genotype influences elite athletic performance, and the association between *ACTN3* genotype and skeletal muscle performance has since been replicated in athletes and non-athlete cohorts. We have also studied the evolution of the R577X allele during human evolution and demonstrated that the null (X) allele has undergone strong, recent positive selection in Europeans and Asian populations. We have developed an *Actn3* knockout mouse model that replicates α-actinin-3 deficiency in humans and has already provided insight into the role of α-actinin-3 in the regulation of skeletal muscle metabolism, fibre size, muscle mass and contractile properties. In particular, mouse muscle lacking α-actinin-3 uses energy more efficiently, with the fast fibers displaying metabolic and contractile properties of slow oxidative fibers. While this favors endurance activities, the trade off is that the muscle cannot generate the rapid contractions needed to excel in sprinting. We propose that the shift towards more efficient aerobic muscle metabolism associated with α-actinin-3 deficiency also underlies the adaptive benefit of the 577X allele. Our future studies will focus on the effect of *ACTN3* genotype on response to exercise and ageing, and the onset and severity of muscle disease phenotype.

Keywords: α-actinin-3, *ACTN3*, skeletal muscle, evolution, athletic performance, positive selection

α-Actinin-3 in Skeletal Muscle

The contractile apparatus of skeletal muscle is composed of repeating units (sarcomeres) that contain ordered arrays of actin-containing thin filaments and myosin-containing thick filaments. The Z lines are electron-dense bands, perpendicular to the myofibrils that anchor the thin filaments. The α-actinins are a major component of the Z line in skeletal muscle, are structurally related to dystrophin, and have a number of functional domains: an N-terminal actin-binding domain, a central rod domain, and a C-terminal region that contains two potential calcium-binding EF hand motifs (Figure 1; Blanchard et al., 1989; Macarthur & North, 2004). In mouse and humans, the *ACTN2* and *ACTN3* genes encode the skeletal muscle α-actinins, α-actinin-2 and α-actinin-3, which differ in sequence adjacent to the actin-binding domain and have high sequence similarity elsewhere (81% identical and 91% similar) (Beggs et al., 1992). α-actinin-2 is present in all human skeletal muscle fibres, and α-actinin-3 is present only in type 2 (fast, glycolytic) fibres (North & Beggs, 1996).

Based on their interaction with many of the structural and signalling proteins associated with the Z-line, especially skeletal α-actin, the α-actinins likely perform a static function in skeletal muscle to maintain the ordered myofibrillar array as well as a regulatory function in coordinating myofiber contraction (reviewed in Macarthur & North, 2004). The α-actinins are thought to maintain muscle cell integrity by linking dystrophin (deficient in Duchenne muscular dystrophy) with the integrin adhesion system (Hance et al., 1999). In vitro studies suggest that the C-terminus of sarcomeric α-actinin plays a crucial role in the maintenance of Z-line integrity and myofibrillar organization (Lin et al., 1998). This region is the site of binding for a number of important interacting

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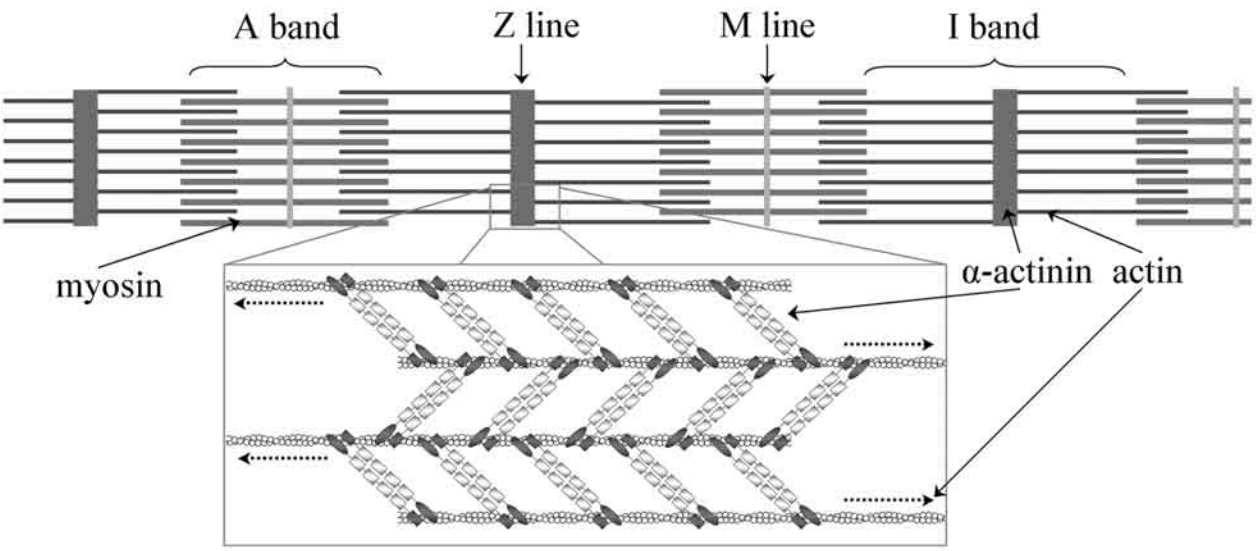

Figure 1

Localisation and domain structure of the sarcomeric α-actinins. The sarcomeric α-actinins are found at the Z line, where they anchor actin-containing thin filaments from adjacent sarcomeres. Antiparallel dimers of α-actinin cross-link actin filaments and stabilize them against force generated by the contractile apparatus (top inset; dashed arrows indicate direction of force). The lower inset illustrates the domain structure of the α-actinins, and their antiparallel alignment in dimeric form. ABD, actin-binding domain; R1-4, spectrin-like repeats 1-4; EF, EF hand region.

Note: Adapted from MacArthur & North (2004).

proteins, including the Z-line protein myotilin (Salmikangas et al., 2003). The α-actinins also bind to the glycolytic enzyme, fructose-1,6-bisphosphatase (Gizak et al., 2003), to glycogen phosphorylase (Chowrashi et al., 2002), and to the calsarcins which bind to calcineurin, a signaling factor which plays a role in the specification of muscle fiber type (Serrano et al., 2001). Thus differential expression of the sarcomeric α-actinins may also influence skeletal muscle metabolism and/or fiber type specification.

Personal Perspective: A False Start

The two human skeletal muscle α-actinin genes were first cloned by Dr Alan Beggs in Boston (Beggs et al., 1992). During my genetics fellowship and post-doctoral training at the Children's Hospital in Boston, one of my projects with Alan was to study the expression of the α-actinin genes in normal and diseased human skeletal muscle. At this stage, dystrophin and subsequently merosin (laminin α2) were the only known muscular dystrophy genes, and many of us in the Genetics Division led by Louis Kunkel (who cloned dystrophin) were working under the assumption that sarcolemmal and sarcomeric proteins that are structurally related to, or that interact with dystrophin were excellent candidates for other autosomal forms of muscular dystrophy. On this basis we studied expression of α-actinin-2 and α-actinin-3 in muscle biopsies from 17 patients with muscular dystrophy and found that four out of 17 were deficient in α-actinin-3 by immunofluorescence and/or Western blot analysis (North & Beggs, 1996). We concluded that α-actinin-3 may be a marker for a subset of patients with muscular dystrophy... but included the caveat that 'it remains to be determined whether the deficiency of α-actinin-3 reflects ACTN3 gene mutations or is a secondary phenomenon' (p. 234). A follow-up study identified additional α-actinin-3-negative biopsies from neuromuscular patients with known disorders, suggesting that α-actinin-3 deficiency was a secondary phenomenon in these patients (Vainzof et al., 1997).

I returned to Sydney and established the Neurogenetics Research Unit in 1996 with a focus on studying the etiology and mechanisms underlying neuromuscular disease. We screened 267 muscle specimens with a variety of histopathological features. Although all these biopsies contained apparently normal α-actinin-2 expression, deficiency of α-actinin-3 was identified by immunocytochemistry and/or Western blot in 51 out of 267 cases (19%), a finding that was not associated with any particular histopathological or clinical phenotype. To ascertain whether α-actinin-3 deficiency was associated with mutations of *ACTN3*, we focused initially on one consanguineous family with two affected male siblings with congenital muscular dystrophy and complete deficiency of α-actinin-3. Sequencing of *ACTN3* cDNA from the proband identified a C→T transition at position 1747 in exon 16, converting an arginine to a stop codon at residue 577 (R577X). Direct sequencing of genomic DNA from the proband and his affected sibling confirmed homozygosity for the stop codon. To our surprise, subsequent testing of the parents and two unaffected siblings revealed that all these phenotypically normal individuals had the same

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genotype as the proband and were thus homozygous for the *ACTN3* 577X nonsense mutation.

We subsequently analyzed an additional 125 biopsies for which matched DNA samples were available and determined α-actinin-3 expression and *ACTN3* genotype; of the 46 biopsies with α-actinin-3-deficiency, all patients were homozygous for the stop codon at position 577 (577XX). There was no significant difference in the frequency of homozygous null genotypes among patients with dystrophic, myopathic, neurogenic or normal histology (North et al., 1999). These data suggested that hereditary α-actinin-3 deficiency is common and is not associated with an abnormal neuromuscular phenotype. That is, we had found an example of a common null polymorphism which results in the absence of a muscle structural protein in a significant proportion of the population, and were now embarking on a study of normal human variation.

α-Actinin-3 Deficiency is Common in the General Population

To determine the frequency and ethnic distribution of the *ACTN3* 577X allele in the general population, we genotyped an additional 485 DNA samples. The 577X allele was identified in all ethnic populations studied covering the four main diversity groups of Asia/Americas, Australasia, Africa and Europe; the frequency of the 577X null allele differs between human populations, being about 10% in most African populations but close to 50% in most Eurasian populations (North et al., 1999). We estimate that roughly 16% of the world population have congenital deficiency of α-actinin-3 (that is, over 1 billion people worldwide).

α-Actinin-2 and α-actinin-3 have high sequence homology and form homodimers and heterodimers in vitro and in vivo (Chan et al., 1998) and it is likely that α-actinin-2 is able to 'compensate' for the absence of α-actinin-3 in fast (type 2) fibres in humans. This raised the possibility that *ACTN3* is a functionally redundant gene 'on its way out'. However, subsequent data from our laboratory suggested that *ACTN3* has function(s) independent of *ACTN2* in human and mouse muscle (Mills et al., 2001). The experimental evidence can be summarized as follows:

1. *ACTN3* is highly conserved during evolution. Sequence comparison of human, mouse and chicken *ACTN* genes suggest that human *ACTN2* and *ACTN3* diverged substantially earlier than 300 million years ago, and the proteins encoded by both have evolved very slowly since their divergence.
2. *ACTN2* and *ACTN3* are differentially expressed, spatially and temporally, during human and mouse embryonic development, and *ACTN2* expression does not completely overlap *ACTN3* in mouse postnatal skeletal muscle. *ACTN3* is the predominant fast fiber isoform in both mice and humans.
3. There is marked variation in the frequency of α-actinin-3 deficiency in different ethnic groups. 25% and 18% of people with Asian and European ancestry, respectively, do not express α-actinin-3 (homozygous null for 577X) compared to less than 1% of a South African Bantu population. This raised the possibility that *ACTN3* genotype confers a fitness advantage to humans under certain environmental conditions or at the extremes of performance.

ACTN3 Genotype Influences Human Muscle Performance

The force-generating capacity of type 2 (fast) muscle fibres at high velocity and the capacity of the individual to adapt to exercise training are strongly genetically influenced (Simoneau & Bouchard, 1998; Rankinen et al., 2006). In addition there appears to be an evolutionary 'trade-off' in performance traits for speed vs. endurance activities and this imposes important constraints on the evolution of physical performance in humans and other vertebrates (Garland et al., 1990; Van Damme et al., 2002); that is, an individual is inherently predisposed towards being either a 'sprinter' or a 'stayer'. Thus, we hypothesized that *ACTN3* genotype may be one of the factors that influences normal variation in muscle function. Since any effect on muscle function will be most readily observable at the extremes of human performance, we collaborated with the Australian Institute of Sport to study *ACTN3* genotypes in elite athletes.

We genotyped 429 elite Caucasian athletes from 14 different sports and compared them to 438 healthy Caucasian controls. Athletes were defined as 'elite' if they had represented Australia in their sport at the international level; 50 of the athletes had competed at an Olympic level. There was considerable heterogeneity in *ACTN3* genotype frequencies between groups of athletes from different sports and there was no significant difference between genotype frequencies in the elite athlete group as a whole, compared to controls ($\chi^2 = 5.16$, $p = .26$).

Given the localization of α-actinin-3 to fast skeletal muscle fibers, we hypothesized that its absence would reduce performance in sprint/power events and would therefore be less frequent in elite sprint/power athletes. To test this hypothesis we analyzed genotypes of a subset of 107 elite athletes in our cohort, classified a priori as specialist sprint/power athletes by the Australian Institute of Sport, blinded to genotyping results. For comparison we analyzed a subset of 194 subjects classified independently as specialist endurance athletes. Thirty-two power athletes and 18 endurance athletes had competed at the Olympic level. Remarkably, all the sprint/power athletes had similar genotypic profiles with an extremely low frequency of XX (α-actinin-3 null; 5% compared to 18% in controls) and a high frequency of RR (50% compared to 30% in controls ($\chi^2 = 19.70$, $p < .0001$)). The genotype effect was more marked in females and in sprint/power Olympians (25 male and 7 female),

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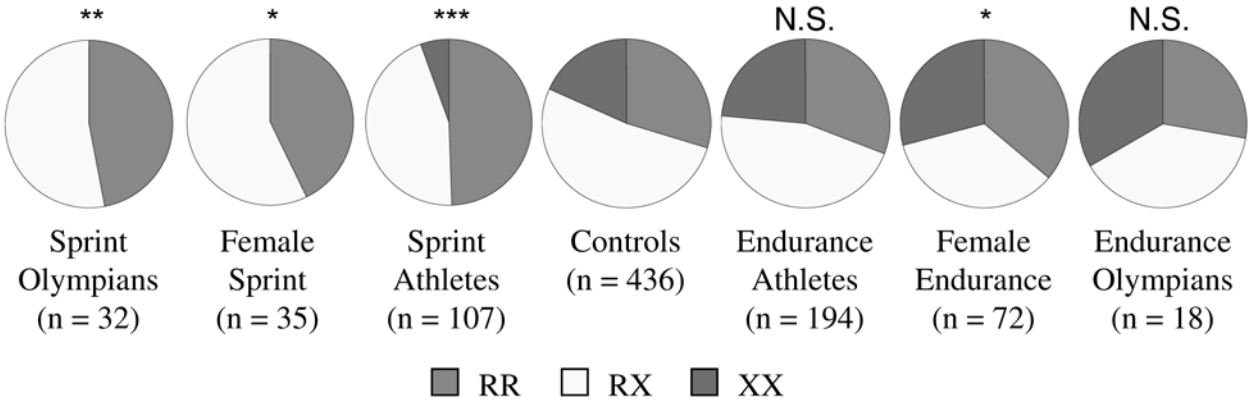

Figure 2

Frequencies of the three ACTN3 R577X genotypes in controls and elite athletes. The sample size in each group is shown. Female athletes and athletes who had competed at an Olympic level are shown as separate groups for both power/sprint and endurance athletes. Significant differences from genotype frequencies in controls (by a c2 test for homogeneity of genotype frequencies between groups) were seen for total power athletes ($P < .0001$), female power athletes ($P < .05$), power Olympians ($P < .01$) and female endurance athletes ($P < .05$).

Note: Adapted from MacArthur & North (2004).

none of whom were XX (Figure 2). In contrast, elite endurance athletes had higher frequencies of XX genotype compared to controls, although the effect was significant only for females ($\chi^2 = 6.15$, $p < .05$). Importantly, genotype profiles in power and endurance athletes deviated in opposite directions and differed significantly from each other ($\chi^2 = 19.45$, $p < .001$), explaining the lack of allelic association when the entire elite athlete cohort was compared to the controls (Yang et al., 2003).

This association between *ACTN3* genotype and muscle performance has been independently replicated. In cohorts from Finland and Greece, sprint athletes had lower 577XX genotype frequency than controls, whereas the frequency of α-actinin-3 deficiency (577XX) was higher in endurance athletes than controls — similar to our findings in Australian athletes (Niemi & Majamaa, 2005; Papadimitriou et al., 2008). None of the Olympic-level sprint athletes tested as part of these studies were deficient in α-actinin-3.

ACTN3 genotype has also been shown to influences muscle performance in nonathletes, with groups demonstrating associations between R577X genotype, muscle strength and response to exercise training in the general population (Clarkson et al., 2005; Delmonico et al., 2007; Vincent et al., 2007). We have also genotyped a large cohort of Greek adolescents and demonstrated that loss of α-actinin-3 is significantly associated with poorer 40 m-sprint performance (Moran et al., 2007; Figure 3). The *ACTN3* R577X polymorphism thus represents an important genetic factor influencing muscle performance in humans.

Recent Positive Natural Selection Has Acted at the *ACTN3* Locus

The athlete and population association studies, along with the varying distribution of 577X allele frequency among different ethnic groups (North et al., 1999), raised the possibility that 577X has been positively selected in some populations. To explore the selective forces that have acted on the R577X polymorphism during human evolutionary history we analyzed DNA sequence and long-range linkage disequilibrium around R577X in individuals of European, East Asian and African ancestry. Our data suggest that the 577X allele is ancient (> 1 million years old). However, there is unusually low sequence diversity and high long-range linkage disequilibrium amongst X allele-containing haplotypes in Europeans and Asians, consistent with strong, recent positive selection on 577X in these populations — suggesting that the 577X allele provided some sort of fitness advantage to modern humans adapting to the novel Eurasian environment. We estimate that selection on the 577X allele began around 15,000 years ago in Europe and around 30,000 years ago in East Asia (Macarthur et al., 2007).

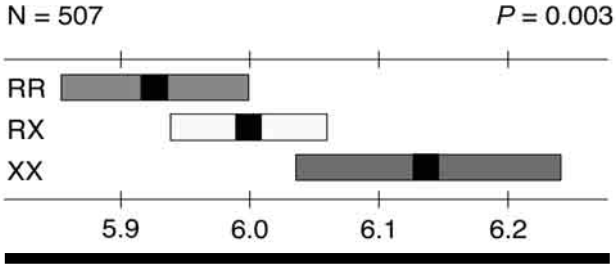

Figure 3

Association of R577X genotype with 40 m-sprint performance in adolescent males. Horizontal axis shows 40 m sprint times in seconds. Black boxes show mean sprint time (back-transformed using the 17/18 year old mean to correct for age differences) and standard deviation, while shaded boxes indicate the 95% confidence intervals, for each genotype in 507 adolescent males. ANOVA analysis revealed a highly significant effect of genotype on sprint time ($P = .003$, fraction of total variance explained by R577X = 2.3%).

Note: Adapted from Moran et al. (2007).

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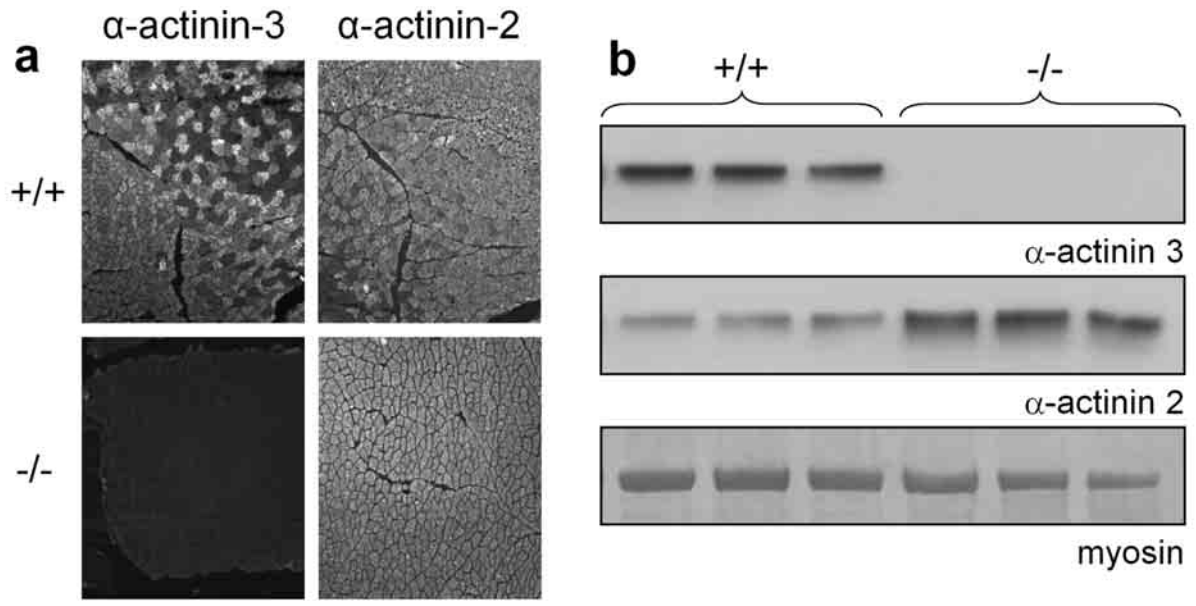

Figure 4

Generation and analysis of *Actn3* KO (-/-) mice. (a) Immunohistochemistry for the sarcomeric α-actinins in WT (+/+) and KO mice, showing loss of α-actinin-3 and altered expression pattern of α-actinin-2 in KO muscle. (b) Immunoblot analysis shows absent α-actinin-3 and up-regulated α-actinin-2 in KO mice compared to WT littermates. Total myosin is shown as a loading control.

Note: From MacArthur et al. (2007).

The *Actn3* KO Mouse Mimics α-Actinin-3 Deficiency in Humans

Concurrently, and to explore the mechanisms by which α-actinin-3 deficiency affects muscle function, we generated and bred mice homozygous for a null allele (targeted deletion of exons 2-7) of *Actn3* (MacArthur et al., 2007). *Actn3* knockout (KO) mice have complete deficiency of α-actinin-3 protein, both by immunohistochemistry (IHC) and Western blotting using an antibody with an epitope at the extreme N-terminus (Figure 4). In wild type (WT) mice, α-actinin-2 is the major fetal isoform and is present in all fiber types during development; α-actinin-3 is not expressed until embryonic day 16 (Mills et al., 2001). Postnatally, α-actinin-3 is the major isoform and is expressed predominantly in fast glycolytic (2B) fibers; α-actinin-2 is expressed predominantly in type 1 and 2A fibers. In contrast, postnatal *Actn3* KO mice express α-actinin-2 uniformly in all fibers, with no apparent loss of 2B fibers; by Western blot, there is an approximately two-fold upregulation of α-actinin-2 expression compared to WT (Figure 4). Thus loss of α-actinin-3 in our KO mouse model results in altered expression of α-actinin-2 so that it is expressed in all fibers — mimicking the expression pattern in humans. Homozygous KO mice are morphologically indistinguishable from WT littermates and display apparently normal sarcomere formation and myofibrillar organization at the light and electron microscope level. Thus α-actinin-2 is at least partially able to compensate for the absence of α-actinin-3 in the muscle of KO mice under baseline physiological conditions.

α-Actinin-3 Deficiency Results in Reduced Strength, Reduced Muscle Mass and Reduced Fast Fiber Diameter in *ACTN3* KO Mice

Given the reduced muscle strength associated with α-actinin-3 deficiency in humans, we examined grip strength in *Actn3* KO mice (Macarthur et al., 2008). All mice produced grip strength values falling within the previously reported normal range; however a 6% to 7% decrease in average grip strength was observed in both male and female KO mice compared to WT (Figure 5a). This reduction in strength is attributable to a reduction in muscle size; all fast KO muscles analyzed, including four separate hindlimb muscles and the spinalis thoracis, weighed significantly less in KO than in WT mice (Figure 5b). The slow soleus muscle and the heart do not normally express α-actinin-3 and thus serve as internal negative controls for the analysis of individual muscles. There was no difference in the weight of the heart of KO mice compared to WT. In contrast to the faster muscles, the soleus in KO mice actually weighed more than in WT mice, possibly a compensatory response to the relative weakness in surrounding muscles. The observed reduction in muscle mass in KO mice is not the result of lower total fiber number or a higher proportion of slow fibers; rather the fast glycolytic 2B fibers (which express α-actinin-3 in WT mice) are substantially reduced in diameter in muscles from KO mice. In the quadriceps, KO muscle fibers expressing myosin heavy chain 2B had an average diameter 34% smaller than 2B-positive fibers in WT muscle (data not shown). In support of this finding, recent work indi-

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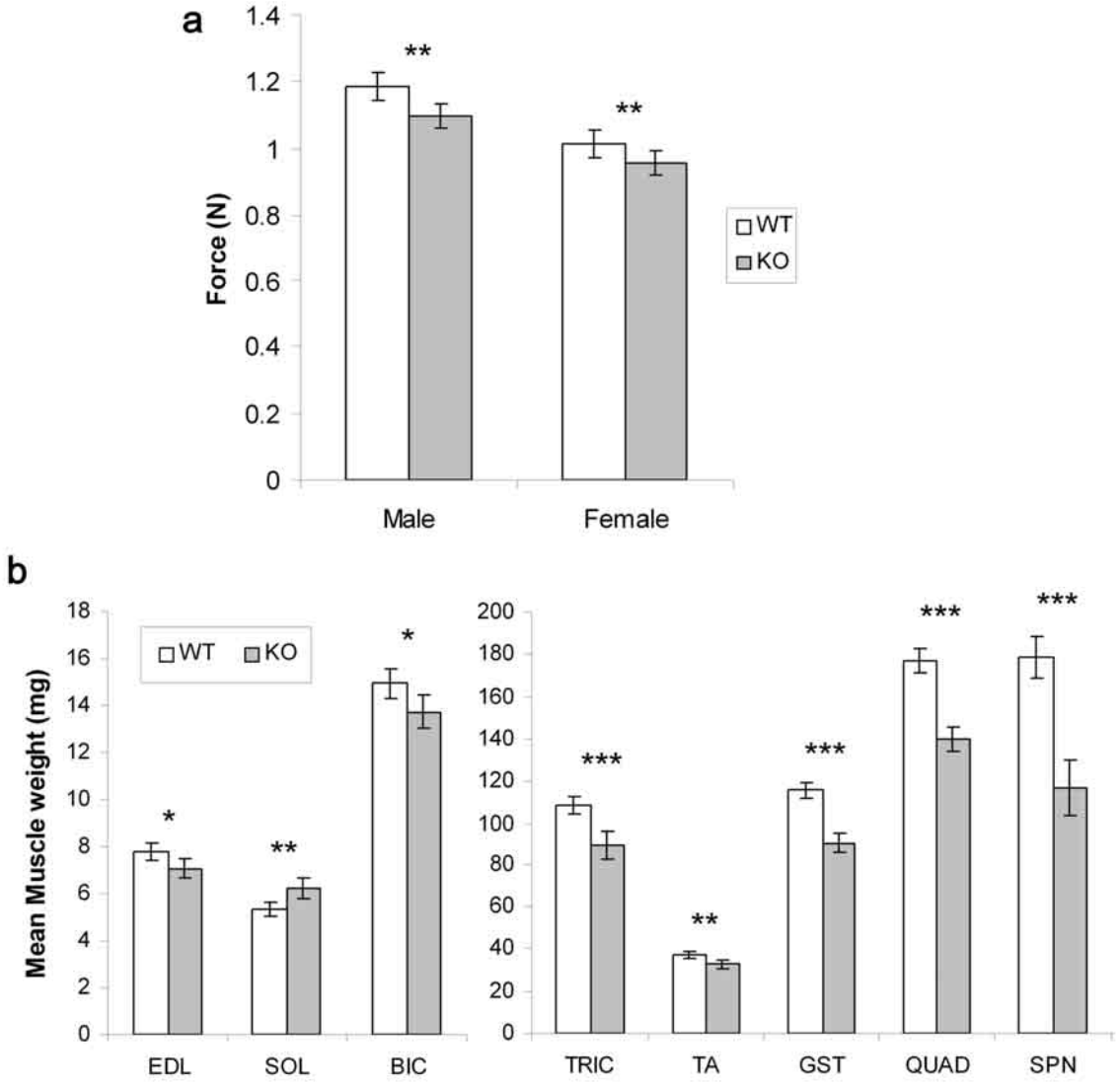

Figure 5

Reduced grip strength and muscle weight in α-actinin-3 deficient mice. (a) KO mice display reduced grip strength compared to WT. Grip strength assays were performed on 7- to 9-week-old male (WT $n = 19$, KO $n = 24$) and female (WT $n = 39$, KO $n = 44$) mice. (b) Mean muscle mass of extensor digitorum longus (EDL), soleus (SOL), medial biceps (BIC), triceps (TRIC), tibialis anterior (TA), gastrocnemius (GST), quadriceps (QUAD) and spinalis thoracis (SPN) excised from male eight week old mice. Note that the three smallest muscles are shown with a separate axis to increase visual clarity. For BIC and TRIC, WT $n = 9$, KO $n = 7$; for all other muscles, WT $n = 18$, KO $n = 13$. Data shown as mean ± 95% CI.

Note: $^{*}P < .05$; $^{**}P < .01$; $^{***}P < .001$. From MacArthur et al. (2008).

cates that α-actinin-3 deficiency also reduces the total muscle cross-sectional area occupied by fast (type 2X) fibers in XX humans (Vincent et al., 2007).

α-Actinin-3 Deficiency Results in a Shift in Muscle Metabolism Towards the Aerobic Pathway in *Actn3* KO Mice

Given the specific expression of α-actinin-3 in type 2 (fast) fibers, the differing frequencies of the 577XX genotype in sprint and endurance athletes, and the interaction between the α-actinins and a number of metabolic factors, we investigated whether the loss of α-actinin-3 resulted in alterations of skeletal muscle metabolism. Quadriceps muscle sections from KO mice show more intense staining for markers of aerobic metabolism, NADH and succinate dehydrogenase (SDH), than WT mice (Figure 6), particularly in fast fibers. The increase in staining does not result from a loss of fast fibers, but rather from a switch in the metabolism of these fibers towards the aerobic pathway. Immunoblotting with two mitochondrial proteins (the electron transport chain component cytochrome c oxidase and the outer membrane protein porin) demonstrate an up-regulation of both markers in the muscle of KO mice compared to their WT littermates. We also analyzed the activity of two key enzymes involved in pyruvate metabolism. Pyruvate, the end metabolite of the glycolytic pathway, repre-

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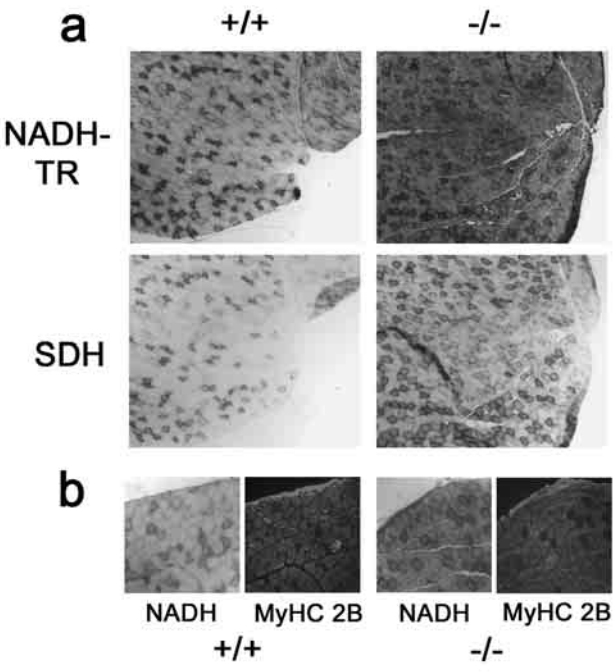

Figure 6

Fast muscle fibers in Actn3 KO mice display increased staining for markers of aerobic metabolism. (a) Representative images of quadriceps muscle sections show increased staining for NADH-tetrazolium reductase (NADH-TR) and succinate dehydrogenase (SDH) in KO muscle compared to WT. (b) Higher-power images of serial sections stained for myosin heavy chain (MyHC) 2B and NADH show that fast glycolytic (2B-positive) fibers stain more darkly for NADH-TR in KO muscle.

Note: From MacArthur et al. (2007).

sents a branch point in muscle metabolism: in fast muscle fibres pyruvate is predominantly converted to lactate by the anaerobic enzyme lactate dehydrogenase (LDH), while in slow fibres it is preferentially oxidized within the mitochondria, a slower but more efficient process in which citrate synthase (CS) catalyzes a rate-limiting step. The activity of LDH is 16% lower ($P < .001$) and that of CS is 22% higher ($P < .05$) in KO mice compared to WT (Macarthur et al., 2007).

We have extended our metabolic analysis to include a panel of enzymes representing a much broader sample of the metabolic network (Figure 7; Macarthur et al., 2008). The glycolytic pathway showed variable changes, with substantially increased activity for hexokinase (26%) and glyceraldehyde-6-phosphate dehydrogenase (62%) in KO mice, but no detectable change in the activity of phosphofructokinase (PFK). Two mitochondrial enzymes involved in fatty acid oxidation, beta hydroxyacyl-CoA dehydrogenase (bHAD) and medium chain acyl-CoA dehydrogenase (MCAD), also showed 30–42% higher activity in KO muscle, suggesting an increased reliance on oxidation of fatty acids in the absence of α-actinin-3 expression. In combination these data indicate a shift in the muscle metabolism of fast fibers in KO mice away from their traditional reliance on anaerobic metabolism towards the aerobic pathway.

To determine if these metabolic changes are associated with changes in the physiological properties of skeletal muscle, we studied isolated extensor digitorum longus (EDL: fast muscles) from KO and WT mice. α-Actinin-3-deficient EDL muscles display significantly longer twitch half-relaxation times (time taken for muscles to relax to half of peak twitch force after a contraction) — 15.8 ± 0.6 ms in KO vs. 13.2 ± 0.6 ms in WT mice ($p = .007$). KO mouse muscles also demonstrate significantly better recovery from fatigue than α-actinin-3-positive muscles ($p < .01$) (Macarthur et al., 2008; Figure 8).

α-Actinin-3 Deficiency Results in Increased Endurance Performance in *Actn3* KO Mice

To determine if the alteration in skeletal muscle metabolism influences endurance performance, we performed an exercise capacity test on *Actn3* KO and WT mice in which the mice were run on a motorized inclined treadmill at increasing speeds until exhaustion. *Actn3* KO mice performed significantly better, running on average 33% further than their *Actn3* WT littermates before reaching exhaustion (Macarthur et al., 2007; Figure 9). These findings suggest that the shift towards oxidative metabolism observed in *Actn3* KO mice increases their intrinsic endurance capacity.

In combination, our data indicate that the loss of α-actinin-3 results in a significant shift in the metabolic pathways of fast muscle fibers, away from their traditional reliance on the anaerobic lactate pathway towards the aerobic pathway normally associated with slow muscle fibers. This is associated with a shift towards the slow phenotype in terms of reduced fiber diameter and contractile properties and is consistent with the association between 577XX genotype and poorer sprint performance in human studies of athletes and nonathletes. The increase in the half-relaxation time in α-actinin-3-deficient muscle would hamper the ability to generate rapid and repetitive forceful contractions required for sprint or power performance. The increased fatigue resistance of KO mice is consistent with improved endurance performance in XX humans. From an evolutionary point of view, our finding that α-actinin-3 deficiency increases the metabolic efficiency of skeletal muscle may explain the apparent benefit of the 577X null allele in modern humans adapting to the Eurasian environment — that is, homozygosity for 577X may improve resistance to fatigue or famine.

What Now?

The *ACTN3* R577X polymorphism is a common genetic variation in the general population and results in a well-defined molecular phenotype (presence or absence of a muscle structural protein). More than one billion individuals worldwide are deficient in α-actinin-3. Our demonstration of *ACTN3* genotype frequency differences between power and endurance

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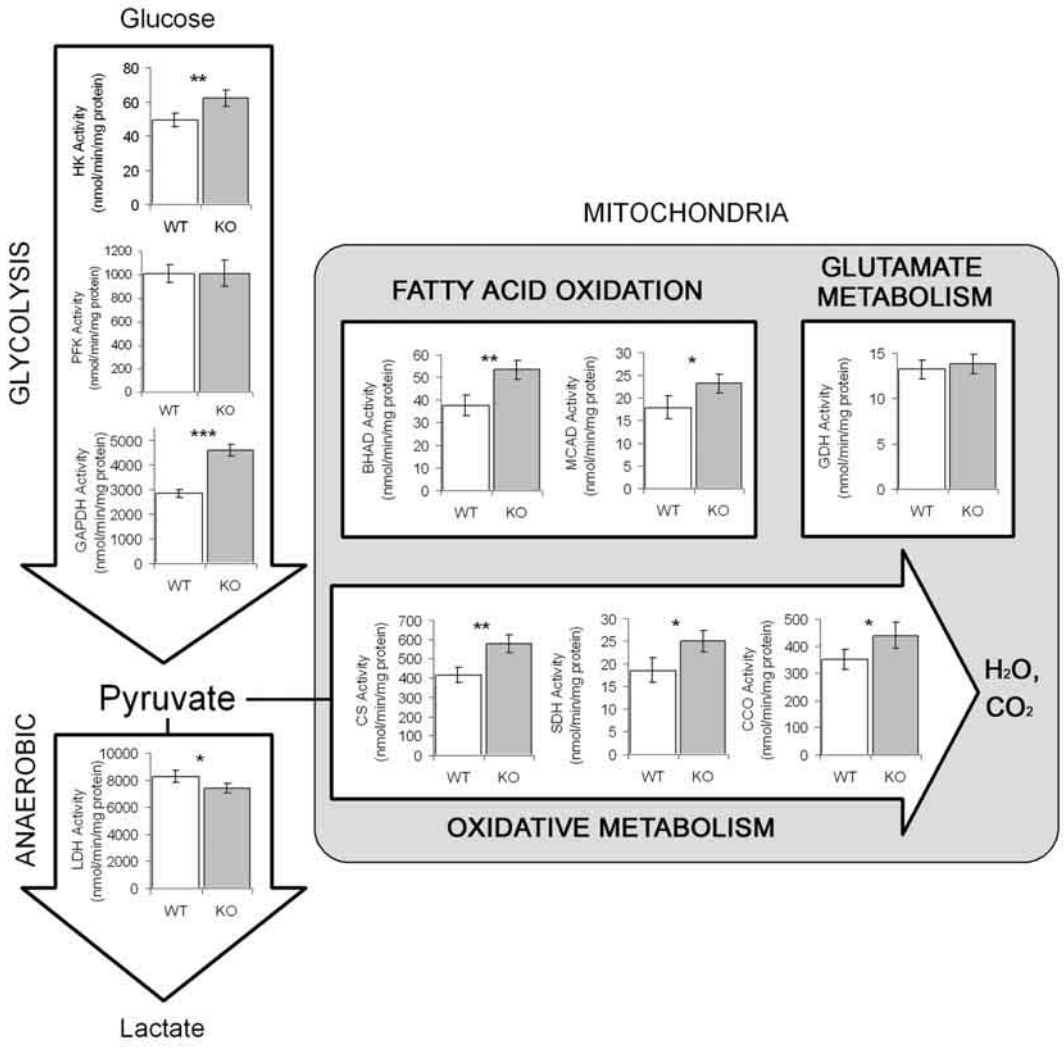

Figure 7

KO mouse quadriceps muscles display a metabolic shift with decreased activity of anaerobic metabolism and increased activity of both glycolytic and mitochondrial aerobic enzymes. Enzyme activities of hexokinase (HK), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), hexokinase (HK), lactate dehydrogenase (LDH), citrate synthase (CS), succinate dehydrogenase (SDH), cytochrome *c* oxidase (CCO), 3-hydroxyacyl-CoA dehydrogenase (BHAD), medium chain acyl-CoA dehydrogenase (MCAD) and glutamate dehydrogenase (GDH) were determined (WT $n = 8$, KO $n = 8$). All graphs show mean ± 95% CI.

Note: $^{*}P < .05$; $^{**}P < .01$; $^{***}P < .001$. From MacArthur et al. (2008).

elite athletes provides a clear example of how positive selection during the course of human evolution contributes to normal variations in contemporary human populations. There are likely to be many genes that contribute to the 'fitness' or 'performance' phenotype — but α-actinin-3 is the first structural protein for which such an association has been demonstrated. In the broader context of molecular evolution, understanding the function(s) of α-actinin-3 will contribute to our understanding of diversity in human populations — with potential public health implications related to variability in metabolic efficiency, and the factors that influence muscle bulk and muscle strength in the general population. To this end we are now studying the effects of *ACTN3* genotype on response to exercise and ageing, and we are further exploring the mechanisms by which the presence or absence of α-actinin-3 influences muscle metabolism.

The metabolic effects of *ACTN3* genotype may also act as a modifier of disease severity. *ACTN3* has recently been shown to alter exercise capacity in McArdles disease (Lucia et al., 2007). The α-actinins also interact with a number of proteins implicated in other forms of inherited muscle disease, such as dystrophin, actin, nebulin and myotilin. We plan to cross our *Actn3* KO with mouse models of Duchenne muscular dystrophy (dystrophin), nemaline myopathy (actin) and myofibrillar myopathy (myotilin) to determine whether the presence or absence of α-actinin-3 influences the clinical or pathological phenotype. Studies in the mouse model will, in turn, guide the specific phenotypes that we explore in humans as we

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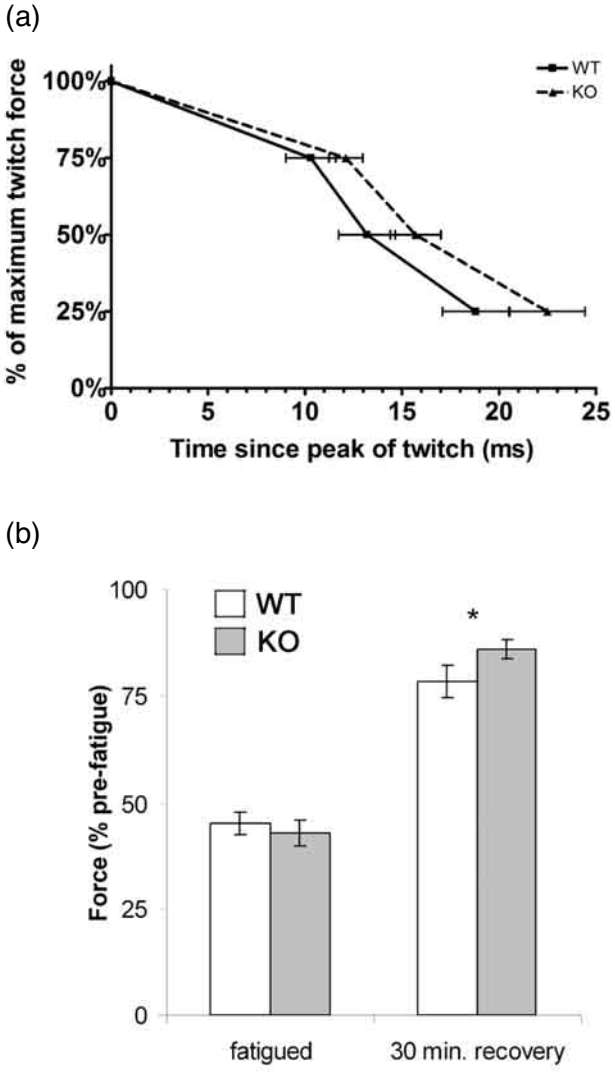

Figure 8

Isolated KO extensor digitorum longus (EDL) displays characteristics of a 'slower' muscle, with (a) longer half-relaxation times (mean ± SEM, WT $n = 8$, KO $n = 10$); and (b) enhanced force recovery following fatigue compared to WT (mean ± 95% CI, WT $n = 6$, KO $n = 8$).

Note: $^*P < .05$. From MacArthur et al. (2008).

dissect the relevance of *ACTN3* genotype to human health and disease.

Those Without Whom None of This Would be Possible ...

The α-actinin-3 project has only been possible because of the hard work and dedication of my lab team and the excellence and enthusiasm of our many collaborators.

Laboratory Team

The laboratory team has been led by Dr Nan Yang over the past 10 years and he and Dr Daniel Macarthur (over 6 years as an Honours student, PhD student and now postdoc) have made a major contribution to our athlete studies, the development of the mouse model and the human evolution studies. Michelle Mills (now Moeskops) was instrumental in the initial studies of α-actinin-3 expression in mouse tissue and during tissue and embryonic development. Jane Seto (PhD student) has made a major contribution to the characterization of the mouse muscle phenotype and is currently studying the effects of α-actinin-3 deficiency on ageing and exercise. Joanna Raftery was pivotal in our extensive sequencing studies for the evolutionary analysis, and establishment of qPCR and microarray analysis of mouse muscle — as well as maintaining and overseeing our extensive mouse breeding program. Dr Kate Quinlan has been responsible for establishing and performing the metabolic analyses and educating us all on the complexities of muscle metabolism, as well as introducing yeast 2 hybrid methodology for further studies of the binding partners of the α-actinins. Dr Mimi Berman (PhD student) is exploring the metabolic consequences of *ACTN3* genotype and Monkol Lek (PhD student)) is focusing on the functional differences between α-actinin-2 and -3.

Collaborators

Alan Beggs at the Children's Hospital in Boston introduced me to the α-actinins. Our ongoing work together aims to determine the differential roles of α-actinin-2 and -3 in skeletal muscle.

Simon Easteal at Australian National University has superb expertise in molecular evolution. He became involved in the project when we initially identified R577X as a polymorphism rather than a mutation and he and Gavin Huttley have made invaluable contributions to our studies of human evolution ever since.

Jason Gulbin, Alan Hahn and Peter Fricker at the Australian Institute for Sport provided access to our athlete cohort and the analysis of sprint versus endurance performance.

Yannis Pitsiladis and his colleagues at the University of Glasgow, Colin Moran and Richard Wilson, have helped us explore the effects of *ACTN3* genotype in nonathletes.

Peter Gunning at the Children's Hospital at Westmead was the source of much wisdom when we first identified R577X as a polymorphism rather than a disease-causing mutation. He convinced me that it was worth pursuing this interesting phenomenon. Since then he and Jeff Hook and Frances Lemckert in his laboratory have made a major contribution to the generation of the KO mouse.

Edna Hardeman at the Children's Medical Research Institute and Anthony Kee and Emma Kettle in her lab have provided invaluable expertise in the interpretation of mouse phenotyping — particularly looking at the response to exercise and initial electron microscopy of muscle ultrastructure.

Stewart Head and Stephen Chan at the University of New South Wales have an ongoing involvement in physiological studies of the KO mouse and have provided

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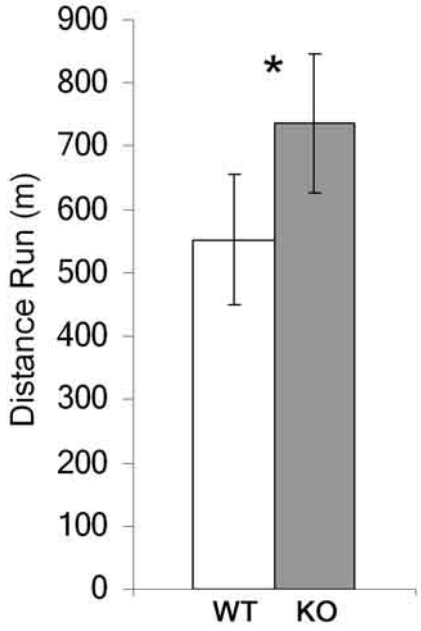

Figure 9

Actn3 KO mice have greater endurance capacity. Graph shows total distance run before exhaustion in an intrinsic exercise capacity test by 20 *Actn3* WT and 23 KO mice. Error bars indicate 95% CI.

Note: *$P < 005$. From MacArthur et al. (2007).

major insights into the changes in contractile properties in response to loss of α-actinin-3.

Gregory Cooney and Nigel Turner at the Garvan Institute have provided us with much needed expertise in studying the metabolic phenotype in the mouse and have been extremely generous with their time, reagents and assistance in performing and interpreting the various assays.

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