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Research Paper

The dependence of preferred competitive racing distance on muscle fibre type composition and *ACTN3* genotype in speed skaters

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It is generally accepted that muscle fibre composition may influence physical performance. The α-actinin-3 (*ACTN3*) gene R577X polymorphism is suspected to be one of the contributing gene variations in the determination of muscle fibre type composition and athletic status. In the present study, we examined the dependence of average preferred racing distance (PRD) on muscle fibre type composition of the vastus lateralis muscle in 34 subelite Russian speed skaters (20 men and 14 women) who competed in races of different length (500–10,000 m). We also investigated the association between the *ACTN3* polymorphism and muscle fibre characteristics in 94 subjects (60 physically active healthy men and 34 speed skaters), as well as the relationship between PRD and *ACTN3* genotype in 115 subelite and elite speed skaters. In addition, *ACTN3* genotype and allele frequencies of the 115 speed skaters were compared with 1301 control subjects. The *ACTN3* XX genotype frequency was significantly lower in sprinters ($n = 39$) compared with control subjects (2.6 *versus* 14.5%; $P = 0.034$). We observed a positive relationship between PRD and the proportion of slow-twitch muscle fibres that was close to linear, but better fitted a logarithmic curve ($r = 0.593$, $P < 0.0005$). The *ACTN3* R577X polymorphism was associated with muscle fibre composition (slow-twitch fibres: RR genotype, 51.7 (12.8)%; RX, 57.4 (13.2)%; XX 61.5 (16.3)%; $\rho = 0.215$; $P = 0.049$) in the overall muscle biopsy group, and with PRD of all athletes ($\rho = 0.24$, $P = 0.010$), indicating that *ACTN3* XX genotype carriers exhibit a higher proportion of slow-twitch fibres and prefer to skate long-distance races. However, the majority of the association between muscle fibre type and PRD was independent of *ACTN3* genotype. In conclusion, the *ACTN3* R577X polymorphism is associated with preferred racing distance in speed skaters and muscle fibre type composition. Thus, it is probably partly via associations with fibre type that the R577X polymorphism contributes to a small but perhaps important component of the ability to perform at a high level in speed skating.

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There is a strong relationship between muscle fibre type distribution and athletic performance. Endurance-oriented athletes are reported to have remarkably high type I (slow-twitch, fatigue-resistant; ST) fibre numbers in their trained muscle groups (Iazvikov *et al.* 1988; Ricoy *et al.* 1998; Andersen *et al.* 2000; Zawadowska *et al.* 2004), whereas muscles of sprinters and weightlifters predominantly consist of IIa/IIx (fast-twitch) fibres (Iazvikov *et al.* 1988; Andersen *et al.* 1994, 2000). The proportion of type I fibres in the most studied human muscle, the vastus lateralis, is typically around 50%, but there are wide variations (range 5–90%; Klitgaard

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et al. 1990; Andersen *et al.* 2000; Andersen & Aagaard, 2000).

On the basis of comparative analyses of fibre type composition in monozygotic and dizygotic twins and other siblings, Simoneau & Bouchard (1995) concluded that the genetic component for the proportion of type I fibres in human muscles is of the order of 40–50%, indicating that muscle fibre type composition is determined by both genotype and environment. The genetic variance is that portion of the individual differences associated with genetic polymorphisms at relevant genes and other DNA regions. It incorporates the effects of single genes, the contribution of polygenes, the genetic–environment interaction effects, and a variety of gene–gene interaction effects. Environmental variance is dependent on factors such as nutritional habits, level of habitual physical activity, non-heritable intrauterine influences, and a variety of other lifestyle components and factors from the social and physical environment (Simoneau & Bouchard, 1995).

The α-actinin-3 (*ACTN3*) gene R577X polymorphism is suspected to be one of the contributing gene variations in the determination of muscle fibre type composition and athletic status. Accordingly, several studies demonstrated that the frequency of the non-functional XX genotype (that indicates an α-actinin-3-deficient phenotype) is lower in elite sprint and power athletes than in control subjects (reviewed by Yang *et al.* 2009). Furthermore, Vincent *et al.* (2007) reported that the percentage cross-sectional area and number of type IIx fibres of vastus lateralis was greater in the RR than the XX genotype group of young healthy men. This relationship corresponds with the function of α-actinins in skeletal muscle fibres. They constitute the predominant protein component of the sarcomeric Z-line, where they form a lattice structure that anchors together actin-containing thin filaments and stabilizes the muscle contractile apparatus (Squire, 1997). Moreover, interacting with many muscle proteins, α-actinins carry out some signalling and metabolic functions, notably interacting with calcineurin, which plays an important role in the determination of muscle fibre type (Olson & Williams, 2000; Berman & North, 2010). Expression of α-actinin-3 is limited to fast skeletal muscle fibres responsible for generating force at high velocity (Mills *et al.* 2001; Vincent *et al.* 2007).

Speed skating includes racing different distances (500–10,000 m) and durations (world records range from ∼34 s to ∼14 min), thus suggesting the predominant use of different types of energy sources and muscle fibres. Some mixture of slow-twitch (to sustain skating posture) and fast-twitch fibres (to effect push off) in the hip and knee extensors seems necessary for optimal skating performance (de Groot *et al.* 1987). Iazvikov *et al.* (1988) reported that sprinters (500 and 1000 m speed skaters) had a higher proportion of type II skeletal muscle fibres (IIa 56 ± 6%; IIb 31 ± 7%), while the muscles of long-distance skaters were predominantly composed of type I muscle fibres (60 ± 4%). The performance in speed skating is largely determined by the external power production of the speed skater. This power is necessary to overcome the air and ice friction and to increase the kinetic energy of the skater (de Koning *et al.* 1992, 1994). van Ingen Schenau *et al.* (1990) have shown that a fast acceleration (high initial power output) is crucial for the sprinting events (500 and 1000 m), while for the long distances the skaters should combine a fast but short-lasting start with a constant power output following the start in order to minimize air frictional losses. The list of determinants of success in speed skating also includes the following factors: increased cross-sectional area of quadriceps femoris muscles (Kanehisa *et al.* 1996); optimal pacing strategy (Muehlbauer *et al.* 2010; Hettinga *et al.* 2011); anthropometric features (shorter legs and longer trunks; Sovak & Hawes, 1987); and increased aerobic capacity (Nemoto *et al.* 1988).

The aim of the present study was to examine the dependence of average preferred competitive racing distance (PRD) on muscle fibre type composition of the vastus lateralis muscle in Russian speed skaters, the association between *ACTN3* polymorphism and muscle fibre composition, and the relationship between PRD and *ACTN3* genotype of speed skaters. In addition, *ACTN3* genotype and allele frequencies of speed skaters were compared with those of control subjects.

Methods

Ethical approval

The study was approved by the Ethic Committee of St Petersburg University and by the Physiological Section of the Russian National Committee for Biological Ethics. Written informed consent was obtained from each participant. The study complied with the guidelines set out in the Declaration of Helsinki.

Study participants

One hundred and fifteen male ($n = 71$; age 22.1 ± 0.7 years; height 179.5 ± 0.7 cm; body mass 75.0 ± 1.1 kg) and female Russian speed skaters ($n = 44$; age 20.8 ± 0.6 years; height 167.6 ± 0.9 cm; body mass 61.1 ± 1.0 kg) of regional or national competitive standard participated in the study. There were 32 athletes classified as 'elite' (having finished in the top eight positions of a major international competition), of whom 12 athletes were 'top elite' athletes (prize winners of the World and European Championships, World Cups and Olympic Games). There were 60 athletes classified as 'subelite' (participants in international competitions).

Table 1. Stratification of 115 speed skaters into nine groups according to the value of average preferred racing distance

Group	*n*	Preferred racing distance (m)	Usual competitive racing distances (m)	Racing time*
I	26	750	500, 1000	34.03–1:06.42
II	13	1000	500, 1000, 1500	34.03–1:41.04
III	12	1250	1000, 1500	1:06.42–1:41.04
IV	7	1833	1000, 1500, 3000	1:06.42–3:37.28
V	17	2250	1500, 3000	1:41.04–3:37.28
VI	17	3167	1500, 3000, 5000	1:41.04–6:03.32
VII	11	4000	3000, 5000	3:37.28–6:03.32
VIII	9	5500	1500, 5000, 10,000	1:41.04–12:41.69
IX	3	7500	5000, 10,000	6:03.32–12:41.69

*Note: world records for men as of July 2011.

Table 2. Characteristics of muscle biopsy groups

	Athletes			
Characteristics	Women	Men	All	Physically active men
n	14	20	34	60
Age (years)	18.2 (1.9)	18.9 (1.7)	18.6 (1.7)	21.3 (2.7)†
Body mass (kg)	59.6 (5.1)	74.5 (3.8)*	67.9 (8.7)	72.9 (8.3)
Height (cm)	168.1 (4.2)	181.2 (4.7)*	176.4 (7.8)	179.2 (5.9)
Body mass index (kg m^{-2})	21.2 (2.1)	22.4 (1.1)	21.9 (1.7)	22.7 (2.6)
Slow-twitch fibres (%)	65.7 (10.5)	64.4 (10.3)	64.9 (10.3)	50.1 (11.1)†
Fast-twitch fibres (%)	40.7 (10.2)	43.1 (9.4)	42.1 (9.7)	52.8 (11.4)†
Cross-sectional area of slow-twitch fibres (μm^2)	5668 (1244)	5599 (1073)	5627 (1129)	5141 (1111)
Cross-sectional area of fast-twitch fibres (μm^2)	5239(1005)	6020(1714)	5699(1497)	5607(1312)

Values are means (SD). *$P \leq 0.0001$, significantly different between female and male athletes. †$P \leq 0.001$, significantly different between physically active men and male athletes.

The other skaters ($n = 23$) were classified as 'non-elite' athletes, being regional competitors with no less than 4 years experience participating in their sports. Each speed skater completed a detailed questionnaire reporting preferred competitive racing distance. An average PRD was determined for each athlete by calculating the mean of the two or three competitive racing distances identified by each athlete (out of 500, 1000, 1500, 3000, 5000 and 10,000 m; note that in Olympic competitions, men do not compete at 3000 m and women do not compete at 10,000 m). The two or three racing distances chosen by each athlete for competition reflect the distance and duration of skating at which they are most successful. Thus, athletes were stratified into nine groups (assigned labels I–IX) according to the value of PRD, covering a spectrum from the more power oriented to the more endurance oriented (Table 1). The athletes of groups I and II (PRD 750–1000 m) were classified as sprinters, the athletes of groups III–VI (PRD 1250–3167 m) as middle distance athletes, and speed skaters of groups VI–IX (PRD 4000–7500 m) were categorized as predominantly endurance athletes.

Control subjects were 1301 healthy unrelated citizens of St Petersburg, Moscow, Naberezhniye Chelny and Surgut (595 men and 706 women; 19.1 ± 0.2 years old) without any competitive sport experience. Geographic ancestry of the athletes and control groups was self-reported. The athletes and control groups were all Caucasians.

Additionally, 60 physically active healthy men and 34 of the 115 speed skaters participated in the study of muscle fibre proportion (for details see Table 2).

Genotyping

Molecular genetic analysis was performed with DNA samples obtained from epithelial mouth cells by alkaline extraction or using a DNK-sorb-A sorbent kit according to the manufacturer's instructions (Central Research Institute of Epidemiology, Moscow, Russia), depending on the method of sample collection (buccal swab or scrape). Genotyping for the C1743T (R577X) variant of the *ACTN3* gene was performed by PCR on a Tercyk thermal cycler (DNA Technology, Moscow, Russia). The PCR primers were forward, CTGTTGCCTGTGGTAAGTGGG, and reverse, TGGTCACAGTATGCAGGAGGG, generating a fragment of 290 bp. The PCR products were digested with *Bst*DE I (SibEnzyme, Novosibirsk, Russia) for 12 h at 60°C and were separated by 8% polyacrylamide gel electrophoresis, stained with ethidium bromide, and

visualized in UV light. All genotyping analyses were conducted blind to subject identity.

Immunohistochemistry

Samples of the vastus lateralis muscle of 94 subjects (speed skaters and physically active healthy men) were obtained with the Bergstrom needle biopsy procedure under local anaesthesia with 1% lidocaine solution. Prior to analysis, samples were frozen in liquid nitrogen and stored at –80°C. Serial sections (10 μm) were prepared using a cryostat and microtome at –20°C, with sections then mounted on slides. The immunoperoxidase technique was employed for immunohistochemical identification of myosin isoforms. Antibodies against the slow (MHCs) and fast myosin isoforms (MHCf) were used [clones NCL-MHCf (a+b) and NCL-MHCs; Novocastra Laboratories, Newcastle, UK]. Sections incubated without primary antibodies were to detect non-specific staining. The antigen–antibody marking was intensified with the Vectastain ABC kit (Vector Laboratories Inc., Burlingame, CA, USA) to visualize the diaminebenzidine peroxidase reaction. Fibre distribution was expressed as the ratio of the number of fibres of each type in a section to the total number of fibres. All fibres (200–300) were measured in each section. The cross-sectional area (in square micrometres) was determined for at least 100 fibres of each type using an image-analysis system (QUANTIMET-500; Leica Microsystems Cambridge Ltd, Cambridge, UK) and a colour digital video camera (JVC TK-1280E; Tokyo, Japan; image resolution 720 × 512 pixels with 8 bits per pixel). Sections used for analysis were all prepared and stained together with Sigma (St Louis, MO, USA) reagents.

Statistical analysis

Genotype distribution and allele frequencies between athletes and control subjects were compared using χ^2 tests. Spearman's or Pearson's correlations (depending on parametricity of data) were used to assess the relationships between the physiological phenotypes (average preferred racing distance, muscle fibre characteristics) and the *ACTN3* genotypes (dummy coded as 1, 2 and 3 for RR, RX and XX, respectively). The squared correlation coefficient R^2 was used as a measure of explained variance. Partial non-parametric correlations (conducted using command syntax in SPSS software) were used to control for the third variable when assessing the relationships between genotype, muscle fibre composition and PRD. Differences in phenotypes between groups (male and female athletes and physically active men) were analysed using unpaired *t* tests. All data are presented as means (standard deviation). Values of $P < 0.05$ were considered statistically significant. Statistical analyses were conducted using GrathPad Instat software (GraphPad Software, Inc., San Diego, CA, USA) and PASW Statistics 18 (SPSS Inc., Chicago, IL, USA).

Results

Preferred racing distance and muscle fibre composition

We found a positive linear correlation between the PRD and the proportion of slow-twitch (ST) muscle fibres ($r = 0.558$, $P = 0.0006$), but the strength of this relationship was increased by fitting a logarithmic curve to the data ($r = 0.593$, $P < 0.0005$; Fig. 1). This relationship indicates that speed skaters with a low proportion of ST fibres are more likely to be successful when skating short-distance races, while speed skaters with a high percentage of slow-twitch fibres in their muscles are specialized in long-distance races. Accordingly, approximately 35% of the variation in PRD could be explained by fibre composition of the vastus lateralis.

ACTN3 genotype, muscle fibre composition and PRD

The *ACTN3* R577X polymorphism was associated both with muscle fibre composition (ST fibres: RR genotype 51.7 (12.8)%; RX genotype 57.4 (13.2)%; XX genotype 61.5 (16.3)%; $\rho = 0.215$; $P = 0.049$) in the overall muscle biopsy group ($n = 94$; Fig. 2) and PRD (RR genotype 1928 (1404) m; RX genotype 2408 (1611) m; XX genotype 3462 (2373) m; $\rho = 0.24$, $P = 0.010$) of all athletes ($n = 115$; Fig. 3), indicating that *ACTN3* XX genotype carriers exhibit a higher proportion of ST fibres and are prone to skate long-distance races. The *ACTN3* genotype explained 4.6% of the variation in muscle fibre composition of the vastus lateralis muscle. It should be noted that heterozygotes (RX) were intermediate between both homozygotes for the proportion of ST muscle fibres and PRD, indicating a co-dominant gene action.

In the smaller group of 34 speed skaters from whom muscle biopsy samples were also obtained, owing to the reduced statistical power, the relationship between *ACTN3* genotype and muscle fibre composition was not statistically significant ($\rho = 0.255$; $P = 0.145$), although the magnitude of correlation was similar to the larger overall biopsy group. In the smaller group of 34 speed skaters, the relationship between ACTN3 genotype and PRD just retained statistical significance ($\rho = 0.339$; $P = 0.050$) and showed a slightly higher magnitude of correlation. In these 34 athletes from whom muscle biopsy samples were also obtained, it was therefore

possible to conduct partial correlations to control for the influence of the third variable when considering the relationships between pairs of the variables genotype, muscle fibre composition and PRD. The strength of the relationship between *ACTN3* genotype and PRD ($\rho = 0.339$) was reduced to $\rho = 0.241$ ($P = 0.177$) when controlling for fibre type composition, suggesting that an important component of that relationship is mediated via the additional relationship between *ACTN3* genotype and muscle fibre composition. However, the relationship between PRD and muscle fibre composition (linearly: $r = 0.558$ and $\rho = 0.592$) was reduced only marginally to $\rho = 0.556$ ($P = 0.001$) when controlling for *ACTN3* genotype, suggesting that the apparent influence of muscle fibre composition on PRD is mostly unrelated to *ACTN3* genotype; indeed, as already stated, our data suggest that only 4.6% of the variation in muscle fibre composition of vastus lateralis can be explained by *ACTN3* genotype. The way in which the data for muscle fibre composition and *ACTN3* genotype contribute to the variability in PRD are summarized in Fig. 4.

Case–control study

The *ACTN3* genotype distributions in the control group (RR genotype 36.8%; RX genotype 48.7%; XX genotype 14.5%) and amongst all speed skaters (RR genotype 36.5%; RX genotype 53.9%; XX genotype 9.6%) were in Hardy–Weinberg equilibrium (control subjects $\chi^2 = 0.437$, $P = 0.804$; athletes $\chi^2 = 1.49$, $P = 0.473$).

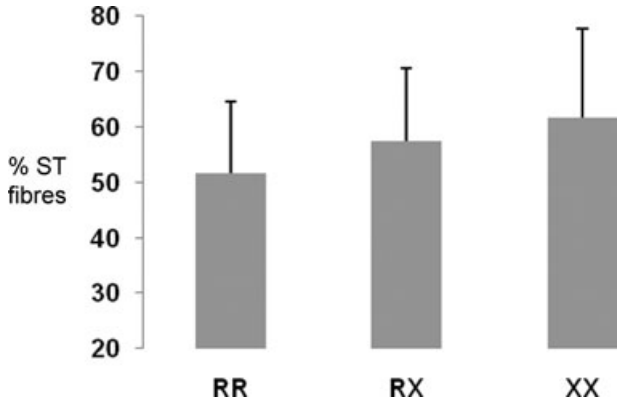

Figure 2. The average percentage of ST fibres in subjects ($n = 94$) with different *ACTN3* genotypes
RR genotype 51.7 (12.8)%; RX genotype 57.4 (13.2)%; XX genotype 61.5 (16.3)%; $\rho = 0.215$; $P = 0.049$. Values are means (SD).

There were no significant differences in *ACTN3* genotype and allele frequencies between men and women amongst athletes and control subjects (data not shown).

Genotype distribution ($P = 0.298$) and the frequencies of the *ACTN3* XX genotype ($P = 0.147$) and X allele (36.5 *versus* 38.9%; $P = 0.524$) in the whole cohort of athletes did not show significant differences when compared with control subjects. However, when considering the distance raced by the speed skaters, the frequencies of the *ACTN3* XX genotype (2.6%, $P = 0.034$) and the X allele (25.6%, $P = 0.024$) were significantly lower in sprint-oriented

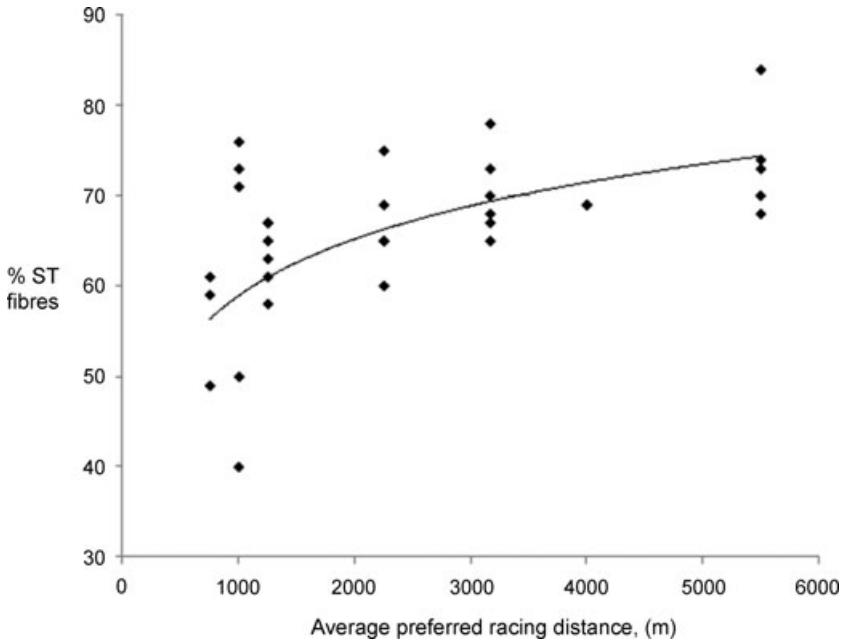

Figure 1. Relationship between proportion of slow-twitch (ST) muscle fibres and average preferred racing distance in speed skaters
$n = 34$, $R^2 = 0.352$, $P < 0.0005$.

athletes (groups I and II; $n = 39$) compared with control subjects. Furthermore, analysis revealed a linear trend of increasing *ACTN3* XX genotype frequency with skating distance with three major groups (sprinters 2.6%; middle-distance athletes 11.3%; endurance-oriented speed skaters 17.4%; $P = 0.046$ for linear trend; Fig. 5).

Additional analysis showed no association between the *ACTN3* polymorphism and competitive standard achieved by the athletes, either as a whole group or when considered as sprint-oriented or endurance-oriented subgroups (data not shown).

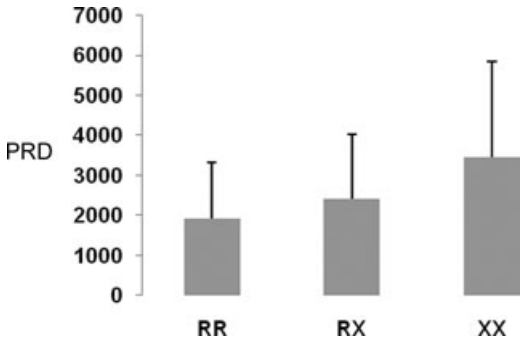

Figure 3. The average preferred racing distance (PRD) in speed skaters ($n = 115$) with different *ACTN3* genotypes
RR genotype 1928 (1404) m; RX genotype 2408 (1611) m; XX genotype 3462 (2373) m; $\rho = 0.240$, $P = 0.010$. Values are means (SD).

Discussion

In the present study, we have demonstrated that athletes with a high proportion of slow-twitch fibres are more successful when skating long races, while speed skaters with a high percentage of fast-twitch fibres in their muscles are more successful when skating short-distance races. These results demonstrate that data from the previous study of Russian speed skaters (Iazvikov *et al.* 1988) are still applicable today, despite advances in various aspects of preparation of athletes for elite competition. Other data from athletes in other sports also indicate that muscle fibre type proportion influences physical performance capability (Andersen *et al.* 1994; Ricoy *et al.* 1998; Andersen & Aagaard, 2000; Zawadowska *et al.* 2004). We have shown that muscle fibre composition can explain approximately 35% of the variation in PRD in speed skaters, suggesting a substantial impact of the proportion of different muscle fibres on competition specialty of speed skaters.

Genetics has a substantial influence over components of athletic performance such as strength, power, endurance, muscle fibre composition, flexibility, neuromuscular co-ordination, temperament and other phenotypes. Accordingly, athletic status and muscle fibre composition are heritable traits; around 66 and 45% of the variances in athletic status and muscle fibre composition, respectively, are explained by genetic factors (Simoneau & Bouchard, 1995; De Moor *et al.* 2007). The *ACTN3* gene R577X polymorphism is suspected to be one of the contributory gene variations in the determination of muscle fibre type composition and athletic status. Our data show statistically significant, though rather weak, relationships between *ACTN3* genotype and both muscle fibre composition and PRD. In fact, more than half of the association between *ACTN3* genotype and PRD seems to be mediated via muscle fibre composition (Fig. 4). However, the total variability in PRD associated with *ACTN3* genotype, including components mediated via muscle fibre composition (4.6%) and other mechanisms (1.2%), is only 5.8%. Vincent *et al.* (2007) have shown that the percentage cross-sectional area and number of type IIx fibres of vastus lateralis was greater in the RR than the XX genotype group of young healthy men ($n = 44$; aged 18–29 years). Furthermore, Norman *et al.* (2009) found a slightly (but not significantly, owing to small sample size) higher ($39 \pm 14\%$) proportion of type IIa muscle fibres in carriers of the RR genotype in comparison with the RX ($36 \pm 6\%$) and XX genotypes ($31 \pm 15\%$) of 37 young men but not in young women ($n = 26$). It should be noted that due to the limitations of the immunohistochemistry method used in the present study, we could not differentiate subtypes of fast-twitch muscle fibres (IIa and IId/x) separately, as was done by Vincent *et al.* (2007) and Norman *et al.* (2009). Even so, these previous data agree with our observed

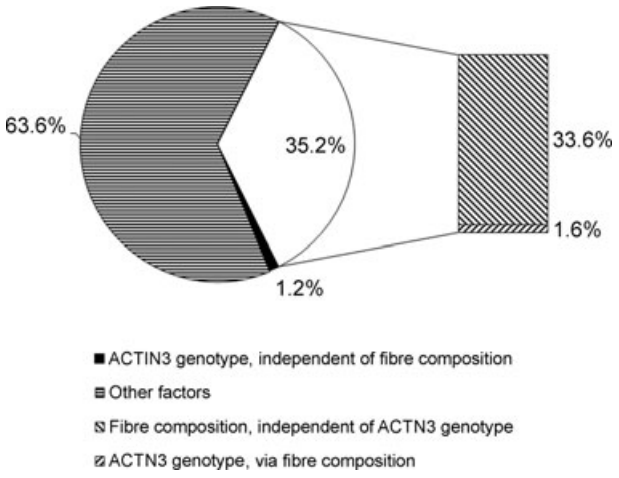

Figure 4. Factors associated with average PRD in speed skaters
Thirty-five per cent of the variability in PRD associated with fibre type (white area in pie chart) includes only a small component associated with *ACTN3* genotype. At the same time, more than half of the association of *ACTN3* genotype with PRD is mediated via fibre composition (1.6 *versus* 1.2% of total variability in PRD).

relationship between the *ACTN3* R577X polymorphism and muscle fibre composition in 94 subjects (athletes and physically active men), indicating that *ACTN3* XX genotype carriers do indeed possess a higher proportion of slow-twitch fibres. Nevertheless, given the substantial heritable component of muscle fibre composition, there are clearly more (and probably many more) genetic variants associated with muscle fibre composition that need to be identified and the findings replicated.

Significant progress has been made during the past few years in the identification of the signalling 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/nuclear factor of activated T-cells (NFAT), peroxisome proliferator-activated receptor gamma coactivator 1 (PGC-1)/peroxisome proliferator-activated receptor delta (PPARδ), calcium–calmodulin-dependent protein kinase and histone deacetylases, thyroid hormone and other pathways (Liu *et al.* 2005; Arany, 2008; Simonides & van Hardeveld, 2008; Schiaffino, 2010). It can be suggested that DNA polymorphisms which influence gene expression of these signalling 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 myosin heavy chain isoforms of adult skeletal muscle. Indeed, several polymorphisms of genes involved in the calcineurin/NFAT pathway, mitochondrial biogenesis, glucose and lipid metabolism, cytoskeletal function, hypoxia/angiogenesis and circulatory homeostasis are reported to be candidate genetic markers for determination of muscle fibre composition (Zhang *et al.* 2003, Ahmetov *et al.* 2006, 2008, 2009*a*,*b*; Vincent *et al.* 2007). One possible explanation for the relationship between α-actinin-3 deficiency (*ACTN3* XX genotype) and slow-twitch muscle fibre phenotype observed previously, and confirmed in the present study, could be evidence that α-actinins interact with signalling proteins, such as calcineurin (reviewed by Berman & North, 2010). Importantly, calcineurin is known to play a key role in the determination of muscle fibre type and muscle hypertrophy (Olson & Williams, 2000). A mechanistic link in the association between *ACTN3* genotype, human performance and muscle characteristics was also proposed in several studies with the use of animal models. MacArthur *et al.* (2007, 2008) reported that the loss of α-actinin-3 expression in a knockout mouse model results in a shift in muscle metabolism towards the more efficient aerobic pathway and an increase in intrinsic endurance performance. Furthermore, Quinlan *et al.* (2010) have shown that α-actinin-3 regulates glycogen phosphorylase activity and calcium handling in mouse myoblasts.

Regarding the 'extended phenotype' of elite sports performance, several case–control studies have reported that *ACTN3* RR genotype is over-represented or *ACTN3* XX genotype is under-represented in strength/sprint athletes in comparison with control subjects (reviewed by Yang *et al.* 2009). Compatible with these previous observations are our present observations that the frequency of XX genotype is significantly lower in Russian speed skaters involved in sprint races than in

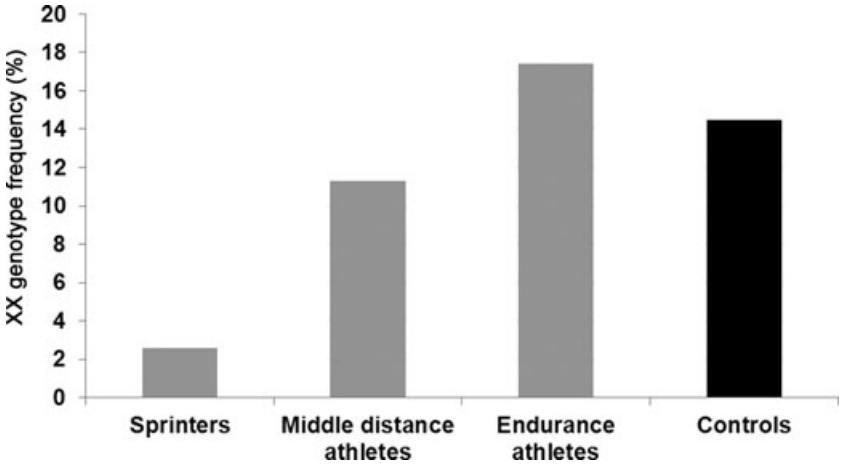

Figure 5. Frequency of *ACTN3* XX genotype amongst speed skaters of different specialties and control subjects
The XX genotype frequency in control subjects was 14.5%. By comparison, it was 2.6 (different from control subjects, $P = 0.034$), 11.3 and 17.4% for sprinters, middle-distance athletes and endurance-oriented speed skaters, respectively ($P = 0.046$ for linear trend). Sprinters are defined as speed skaters of groups I–II (PRD 750–1000 m); middle-distance athletes, speed skaters of groups III–VI (PRD 1250–3167 m); and endurance-oriented athletes, speed skaters of groups VII–IX (PRD 4000–7500 m).

control subjects, there is a linear relationship between XX genotype frequency and PRD in speed skaters, and there is a positive relationship between the number of X alleles possessed and the average preferred racing distance. In the present study, however, *ACTN3* genotype was not related to the competitive standard achieved by the athletes, either as a whole group or when considered as sprint-oriented or endurance-oriented subgroups. These data do not support the notion of selecting young athletes for likelihood of future success in sport based on this single genetic marker. In future, however, when considering several other relevant polymorphisms simultaneously, *ACTN3* may be one of a 'panel' of genetic markers that might provide useful predictions of the possibility of success in particular kinds of sport competitions.

In conclusion, we have demonstrated that athletes prefer to skate particular race distances (based on their empirical observations and likelihood of success in competition) in accordance with their muscle fibre composition, and that muscle fibre composition partly depends on *ACTN3* R577X genotype.

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