ACTN3 R577X Gene Variants Influence Sprint Performance: A Multi-Cohort Study

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30:4; 57-63, 2015

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ABSTRACT

Earlier studies on the contribution of specific genes to sprint performance have been limited by small cohorts from mixed sport disciplines, most lacking quantitative measures of performance. To examine the association between ACTN3 gene variants and sprint times in elite athletes, 555 personal best 100m, 200m, and 400m times by 346 top Caucasian and African origin sprinters from 10 countries were collected. The sprinters were genotyped for ACTN3 R577X variants. On average, male Caucasian sprinters with the ACTN3 577RR variant had faster personal best 200m times than their ACTN3 577XX counterparts and no cases of ACTN3 577XX were found to have achieved the qualifying time for the 2012 Olympic Games (vs. 12 qualified sprinters with 577RR or 577RX genotypes). Using genetic models, it was found that the ACTN3 577R allele dominant model accounts for 0.92% of 200m performance at the elite level. Although sprint performance relies on many gene variants, environment and other factors, the percentage variance in elite level performance explained by the ACTN3 gene is substantial. This project was winner of the Open category in the 2014 European Athletics Innovation Awards and the article published here is adapted from an article previously published in BMC Genomics.

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Introduction

Fifty thousand pairs of eyes are fixed on eight of the fastest men on earth crouched at the starting line of the 200m final at the IAAF World Championship in Athletics in Beijing. The crack of the gun echoes in the warm evening air, and the crowd roars as the competitors leap from their blocks. Just 19.55 seconds later the winner streaks past the finish line. It is Usain Bolt, a 28-year-old from Jamaica. We might ask, why is Bolt, and not Justin Gatlin from USA who finished second in 19.73 seconds or any of the other finalists, the fastest man on earth?

The answer, of course, is a complex one, touching on a million details. Although the likelihood of an individual becoming an elite athlete or succeeding at the very highest level is influenced by genetic factors[1,2] only a handful of genes have been associated with sprint/power performance. Currently the most promising of these candidate genes is the ***ACTN3,*** which encodes the sarcomeric protein α-actinin-3 in skeletal muscle fibres[3]. The expression of α-actinin-3 protein is almost exclusively re-

stricted to fast, glycolytic, type 2X fibres, which are responsible for producing 'explosive', powerful contraction[4]. Homozygosity for common null single nucleotide polymorphism (577XX, rs1815739) in the *ACTN3* gene results in complete deficiency of the α-actinin-3 protein in an estimated 18% of humans worldwide[5], and the *ACTN3* RR genotype has been associated with elite sprint/power athletic performance in several independent cohorts of elite athletes[2].

A higher frequency of the *ACTN3* 577RR genotype (and lower frequency of the α-actinin-3 deficient, 577XX genotype) in elite sprint/power athletes (i.e., sprinters, jumpers, and throwers) was originally found in a case (athletes): control (non-athletes) association study with Australian subjects[3]. This finding was independently replicated in Finnish[6], Greek[7], Russian[8], Israeli[9] and Japanese[10] national/international level athletes. No Olympic-level sprinter has yet been identified with the 577XX genotype[3,7,9,11]. Taken together, these association studies suggest that α-actinin-3 deficiency is detrimental to optimal fast muscle function at the extremes of sprint and power performance. In support of this, mice lacking α-actinin-3 (*ACTN3* knockout mice, mimic the 577XX genotype in humans) demonstrate a shift in the physiological and metabolic properties of 'fast' glycolytic muscle fibres (type 2B) towards a slower, oxidative muscle phenotype (types 1 and 2A), which are responsible for postural and endurance activities[12] and *ACTN3* 577XX humans show lower proportion of fast-twitch muscle fibres[13,14] and lower levels of testosterone[15].

One of the limitations of most of the above studies investigating the association between the *ACTN3* R577X genotype and sprint/power performance is the grouping together of sprint and power athletes from mixed sport disciplines and events (e.g., sprinters, jumpers, throwers, swimmers, and team sport athletes). This approach, while understandable given the very low number of world-class sprinters, reduces the accuracy of the phenotype. Furthermore, to date, only one report involving world-class sprinters of African ancestry[11], has examined the association between *ACTN3* R577X polymorphism and athletic status and found that the lack of positive findings was attributable, at least partly, to the very low frequency of the *ACTN3* 577XX genotypes in the African population, which almost eliminates the possibility of detecting an association.

We sought to address these limitations and provide deeper insight into the influence of the *ACTN3* R577X variants on sprint performance by using a quantitative collaborative approach and by studying the influence of genotype on actual sprint performance. Therefore, the aim of the present study was to examine the association between the *ACTN3* R577X variants and personal best times for 100m, 200m and 400m in a performance-homogenous, cohort of elite Australian, Brazilian, Greek, Jamaican, Italian, Polish, Russian, Lithuanian, Spanish and American (United States) sprinters.

Methods

Participants

A total of 555 personal best 100m, 200m and 400m sprint times of 346 elite pure sprinters from Australia, Brazil, Greece, Jamaica, Italy, Lithuania, Poland, Russia, Spain and the United States were analysed (Table 1). The sprinters from Australia, Greece, Poland, Lithuania and Russia were all Caucasians (189 male and 66 female) whereas a total of 91 male Brazilian, Jamaican, Italian, Spanish and US athletes were from African lineage. The sprinters' personal bests (tail wind <2 m/sec when provided) in official competitions were found online (www.iaff.org) or provided by coaches or the athletes themselves and independently corroborated (Table 1).

The subjects' personal best times, grouped according to ethnic-background (Caucasians/African mixed linage athletes were excluded) and event (100m, 200m or 400m), standardised and expressed relative to the current world records and group bests in the events. We included only 'pure' sprinters with times

Table 1: The 100m, 200m and 400m personal best sprint times (average + SD) in males according to ACTN3 R577X genotype distribution

	Caucasian Ancestry			African Ancestry	
Running event/ Genotype	**RR**	**RX**	**XX**	**RR**	**RX**
100m Male	10.55+0.27 (n=35)	10.58+0.33 (n=44)	10.77+0.31 (n=10)	10.26+0.35 (n=22)	10.28+0.30 (n=11)
200m Male	21.19+0.53 (n=35)	21.29+0.61 (n=36)	21.86+0.54* (n=8)	20.53+0.64 (n=23)	20.98+0.72 (n=11)
400m Male	46.90+1.29 (n=44)	47.41+1.43 (n=46)	47.55+1.42 (n=9)	46.49+1.66 (n=18)	47.29+1.69 (n=7)

**200m (RR vs. RX vs. XX) P<0.016*

that were within 15% of the current world record or group best of the examined events. The following records and group bests were used to calculate the inclusion criterion:

- Male sprinters of African ancestry, 9.58 sec in the 100m and 19.9 sec in the 200m - current world record holder: Usain Bolt (JAM), and 43.18 sec in the 400m - current world record holder: Michael Johnson (USA);
- Female sprinters of African ancestry, 10.49 sec in the 100m and 21.34 sec in the 200m - current world record holder: Florence Griffith-Joyner (USA);
- Male Caucasian sprinters, 9.99 sec in the 100m - group best holder: Christophe Lemaitre (FRA), 19.72 sec in the 200m - group best holder: Pietro Mennea (ITA) and 43.45 sec in the 400m - group best holder: Jeremy Wariner (USA);
- Female Caucasian sprinters, 10.77 sec in the 100m – group best holder: Ivet Lalova (BUL), 21.71 sec inb the 200m – group best holder: Marita Koch (GDR) and 47.60 sec in the 400m – world record holder: Marita Koch (GDR).

Genotyping

Genomic DNA was isolated from buccal epithelium, or white blood cells. The Australian, Greek, Italian, Lithuanian, Russian (from St Petersburg) and Spanish sprinters' DNA samples were genotyped using the polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) method as previously described[4].

The DNA samples of the Polish, Brazilian, Russian (from Chelyabinsk), Jamaican and US sprinters were genotyped in duplicates using an allelic discrimination assay on a Step One Real-Time PCR instrument (Applied Biosystems, Carlsbad, California, USA) with Taqman® probes. To discriminate ***ACTN3*** R577X (rs1815739) alleles, TaqMan® Pre-Designed SNP Genotyping Assay was used (assay ID: C_590093_1_), including appropriate primers and fluorescently labeled (FAM and VIC) MGB™ probes to detect the alleles.

Statistical analysis

To compare the sprinters' records between all genotypes we used the one-way analysis of variance (ANOVA). The Tukey's post-hoc test

was used to determine statisticaly significant differences among the genotype groups. The level of significance was set at 0.05. Using the Simple Interactive Statistical Analysis website (SISA; http://home.clara.net/sisa/) genotype interactions on sprint performance were further assessed using correlation analysis as previously described[16].

Briefly, three genetic models (additive model and two dominant models assuming complete dominance of each allele) were tested. The additive genetic model consisted of 0, 0.5 and 1, to represent R allele homozygotes, RX heterozygotes and homozygotes for the X allele, respectively; for the R allele dominant or X allele dominant genetic models, the corresponding values were 0, 0, 1 or 0, 1, 1, respectively. The proportion of the genetic contribution to phenotypic variance explained by each genetic model was estimated by expressing r^2 from the correlation analyses (taken as an estimate of percentage variance explained under the model) as a percentage of the variance explained by genotype effects in the model-free ANOVAs. This proportion was compared for each model to predict the most accurate model tested.

Results

The personal best 100m, 200m and 400m sprint times (±SD), according to the *ACTN3* genotype and distribution, are presented in Table 1.

ACTN3 genotypes influence 200m personal best time in male athletes

In male Caucasian sprinters a significant association was detected between *ACTN3* genotype and 200m personal best. Using Tukey's Multiple Comparison Test both *ACTN3* 577RR, (-0.66, 95% CI -0.20 to -0.12) and 577RX (-0.56, 95% CI -1.10 to -0.02) individuals were significantly faster than 577XX individuals (P<0.05). We found the R allele dominant model (RR/RX vs. XX) had the best fit explaining 9.65% of sprint time (P=0.005), compared to the additive (7.28%, P=0.01) and

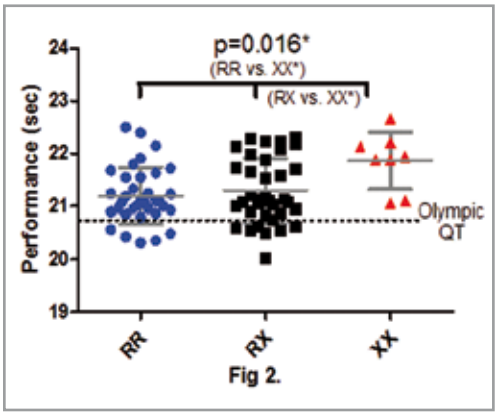

Figure 1: Individual 200m personal best times (±SD) in male Caucasian sprinters according to their ACTN3 R577X genotype and the qualifying times (QT) for the 2012 Olympic Games (20.65 sec).

the X allele dominant model (2.77%, P>0.05) in the correlation analysis. The percentage of the observed variance (coefficient of determination, r^2) explained by the *ACTN3* genotype using this recessive model was 0.92%. The *ACTN3* RR and *ACTN3* RX groups were not significantly different, indicating the presence of one or two R allele does not have a dose dependant effect on 200m personal best time in elite athletes (Figure 1). In elite male African athletes (n=92), there was some evidence for a dose effect of the *ACTN3* R allele and 200m speed (Table 1). Using an unpaired t-test, the *ACTN3* RR individuals had (on average) a faster personal best times than *ACTN3* RX individuals (-0.45, 95% CI, 0.95 to -0.04).

No genotype differences were detected in 100m and 400m sprint performance in both Caucasian and African ancestry sprinters

A trend was observed, but not statistically significant differences in best 100m and 400m times across the *ACTN3* R577X (Table 1). Caucasian females (n=66) were assessed separately and showed similar associations to males, across the genotypes.

Discussion

In this quantitative assessment of genotype with qualifying time in 346 elite sprinters, we have shown that it is rare for humans with the α-actinin-3 deficient (*ACTN3* 577XX) genotypes to reach the standard required to compete in the 200m at the IAAF World Championships in Athletics or Olympic Games. From all the male sprinters' personal best times included in this study, there were no cases of 577XX sprinters who were faster than 2012 Olympic qualifying standard in 200m (20.65 sec). This finding suggests that the *ACTN3* 577XX is detrimental for 200m sprint performance.

In the present study, we have addressed some of the limitations inherent in previous athlete case-control studies regarding the association between *ACTN3* genotypes and elite athletic performance. First, we have studied ten cohorts of elite sprinters, including the fastest sprinters on earth. Consequently, the number of 'pure' elite sprinters (n=346) in the present study is much larger compared to previous association studies, and demonstrates the benefits of a collaborative approach that has been recommended in the field of exercise genomics[17,18,19].

Second, previous reports have grouped together sprint and power athletes from mixed sports disciplines and events[3,7,9,18,10]. Here, we have embraced a more stringent approach and included only elite 'pure' sprinters whose main sporting discipline was the 100m, 200m or 400m. Furthermore, we set explicit criteria (100m, 200m and 400mm best personal running times within 15% from the world record) to ensure a high performance level for the subjects we studied.

Third, we have analysed quantitative measure with respect to *ACTN3* genotypes to assess the genotype effect impact in both males and females separately. Only one previous study has taken a similar approach with 100m sprinters: a subgroup analysis of male Japanese track and field athletes indicated that those harbouring the *ACTN3* 577XX genotype ran the 100m significantly slower than their 577RX and 577RR counterparts[10]. However, this study was limited by its sample size (n=28) and studied only a Japanese cohort.

The analyses we performed show that the male Caucasian sprinters' personal best times are influenced by *ACTN3* genotypes in an event-specific manner. While similar trends were seen in African and female athletes, we highlight that larger cohorts are urgently needed for adequate genotype-performance assessments. Our analyses of male Caucasian performances found that 200m performance (but not 100m and 400m) is influenced by *ACTN3* genotypes. Moreover, we showed that at the highest level of performance (i.e., elite sprinters with personal bests faster than the Olympic qualifying times) there was no significant overlap of the times for each genotype, suggesting that 'every variable counts' for achieving world-class sprinting performance. These findings, together with the separate results from 200m sprints, support the notion that performance in the longer sprinting events (200m, 400m) is influenced by *ACTN3* R577X polymorphism.

These genotype association differences may be related to subtle differences in the physiological performance demands of each event. In the 100m the athlete is required to accelerate for most of the race before reaching their absolute maximum velocity[19]. In the longer sprints the acceleration phase is relatively shorter and it is the ability to maintain the maximum velocity for a longer time period that is the critical factor for top performance[20]. On the one hand, acceleration involves reaction time, the position of the centre of gravity of the body relative to the blocks, stride frequency and stride length, while on the other hand maintaining absolute maximal velocity requires powerful cyclic muscle contractions and efficient utilisation of the energy systems (mostly lactic and 'alactic' anaerobic systems)

that are triggered at different phases of the race[21]. Given the genotype-performance associations at longer sprint distances, this suggests their influence may lie in greater effect on muscle's metabolic potential (switch from P/Cr to lactic anaerobic systems) with repeated powerful contractions.

The *ACTN3* knockout (KO) mouse has provided a possible explanation for the detrimental effect of α-actinin-3 deficient (577XX genotype) on elite sprinting performance. Mechanistic studies in the *ACTN3* KO mouse show that this model mimics *ACTN3 XX in* humans. The wild-type WT mice that express *ACTN3* (equivalent to human RR/RX genotypes) prefer the anaerobic system while *ACTN3* KO mice prefer the aerobic system. Metabolically, the KO mice have significantly higher activity of oxidative enzymes, and lower activity in enzymes of the anaerobic pathway. In addition, enhanced glycogen accumulation due to lower glycogen phosphorylase activity has been observed[22,23] . Their fast fibre properties shift towards a slower metabolic profile which has been linked to increases in calcineurin signalling activity[24] and altered calcium handling[25]. Overall this shift towards a slower physiological and metabolic profile would be detrimental to sprint performance in *ACTN3* 577XX humans[22,12].

Conclusions

Our multi-centre study design has enabled us to gain insights into the effect of *ACTN3* genotypes on elite sprinting performance. *ACTN3* R577X polymorphisms modulate specific sprint phenotypes and influence athletic status at the extremes of human performance. We have shown quantitatively for the first time that the *ACTN3* genotypes account for 0.92% in sprint speed amongst elite male 200m athletes. This difference in performance is substantial and can be the difference between a world record and only making the semi-final at the IAAF World Championships in Athletics or Olympic Games. *ACTN3* R577X polymorphism seems to be more influential on 100m and 400m performance. Despite our findings, the predictive value of these tests remains limited. A substantial amount of performance variation remains unaccounted for and further research into both common and rare variants is still required. With this additional research the findings may have future applications for identifying and coaching talented 200m sprinters.

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