

# The *ACTN3* Gene in Elite Greek Track and Field Athletes

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**Key words**

- gene
- champions
- Greece
- alpha-actinin-3

**Abstract**


The study of genetic influence in the making of an Olympic champion is still in its nascence, but recent work has provided findings regarding the association of the *ACTN3* gene on athletic performance. The aim of this study was to examine genetic differences among elite Greek track and field athletes by analysing a mononucleotide polymorphism in exon 15 of the *ACTN3* gene. Results showed that *ACTN3* genotype and allele fre-

quencies in the top power-oriented athletes were statistically significantly different from those in a representative random sample of the Greek population: the frequency of the RR *ACTN3* genotype in power-oriented athletes vs. the general population was 47.94% vs. 25.97%. This result was even more prominent for comparison of the subgroup of sprinters to controls. The results suggest an overall strong association between the presence of the RR genotype and elite power performance.

**Introduction**


Only a few attempts have been made to shed light upon the influence of genes in making an Olympic champion [7]. The first evidence that a mononucleotide difference in DNA sequence was associated with power ability referred to the R577X polymorphism of the *ACTN3* gene [12]. A transition (C>T) at nucleotide position 1747 in the *ACTN3* coding sequence converts an arginine (R) to a stop codon (X) at residue 577 [5], and this change results in the complete loss of α-actinin-3 protein function in homozygous XX individuals [12]. Alpha-actinin-3 is the most-highly specialised of the four mammalian alpha-actinins, with its expression restricted to type II (fast-twitch) myofibres in skeletal muscle [3,5]. Although it is likely that α-actinin-3 protein has many similar roles to α-actinin-2 [3,5], there is strong evidence to suggest that *ACTN3* gene has been maintained in the genome because of function(s) independent of *ACTN2* [12].

The RR genotype of the *ACTN3* gene has been found to be associated with elite performance in activities requiring bursts of strength in combination with speed (power oriented activities) [6, 12]. The XX *ACTN3* genotype occurs at a higher frequency in some cohorts of elite endurance athletes [2,6]. Additionally, a statistically signifi-

cant difference between endurance athletes and controls was found in females [12], raising the possibility that the total deficiency of the α-actinin-3 protein may confer some beneficial effect on endurance performance. In contrast to the studies mentioned above, there has been recent evidence that an XX *ACTN3* genotype in elite Ethiopians and Kenyan long-distance runners is not associated with endurance performance [13]. To try to further clarify what relevance *ACTN3* polymorphisms might have to performance, we examined their frequency in elite Greek track and field athletes covering a spectrum from power-oriented short distances to endurance-based longer distances. Greeks are southern European, whereas the other two studies are of northern Europeans [6,12]. Thus, studying Greeks gives a more complete picture of European athletes, as European is a generic term for a very heterogeneous population. Therefore, the aim of our study is to elucidate the genetic differences among a group of elite Greek power-oriented track and field athletes and a random representative sample of the Greek population.

accepted after revision  
February 2, 2007

**Bibliography**  
**DOI** 10.1055/s-2007-965339  
 Published online Sept. 18, 2007  
*Int J Sports Med* 2008; 29:  
 352–355 © Georg Thieme  
 Verlag KG Stuttgart • New York,  
 ISSN 0172-4622

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**Table 1** The % of Olympic, European and Balkan level athletes for power oriented and endurance athletes group

Athlete groups	Olympic level	European level	Balkan level	Total
Power-oriented	34%	26%	40%	n = 73
Endurance-oriented	36%	14%	50%	n = 28

## Materials and Methods



### Subjects and controls

The subjects consisted of 101 elite Greek track and field athletes (73 males and 28 females). Athletes were defined as elite and included in the sample if they had been chosen to represent Greece at the international level. Achievements among our sample group included world, European and Balkan records (● Table 1), and many of the athletes were Olympic medallists or finalists and world or European champions. The power-oriented top-level track and field athletes group included 34 sprinters (whose main event was a race of 100 to 400 m), 23 jumpers, 9 throwers and 7 decathletes. The endurance athletes group included 19 long distance runners (whose main event was a race ranging from 3000 m to a marathon length), 4 middle distance runners (whose main event was a race ranging from 800 m to 1500 m length), 3 triathletes (top performers in distance running races) and 2 walkers. The representative random control group was 181 unrelated, healthy, Greek individuals. The birthplaces of the athletes and of the controls were matching in their geographical distribution. All subjects had the Greek nationality and were Caucasians. The study protocol was in accordance with the ethical and moral procedures of the Aristotle University Research Committee.

### Genotyping

Blood samples were obtained from all individuals and DNA was isolated from white blood cells by a standard protocol [4]. The 291 bp fragment of exon 15 of the *ACTN3* gene was amplified by PCR using the forward primer 5'-CTGTTGCCCTGTGGTAAGTGGG-3' and the reverse primer 5'-TGTCACAGTATGCAGGAGGG-3' as recommended by Mills et al. [5]. PCR reaction mix contained 1 µl (30–50 ng) DNA, 0.2 µl primers (100 pmol/µl), and 0.15 µl Taq Polymerase (5 Units/µl). PCR was performed for 30 cycles (30 s each of denaturation at 94 °C, annealing at 58 °C, and extension at 72 °C).

The amplified PCR fragments were subsequently digested with *Dde*I endonuclease (New England Biolabs, Beverly, MA, USA), and the alleles 577R and 577X were distinguished by the presence (577X) or absence (577R) of a *Dde*I restriction site. Digestion of PCR products of the 577X allele yields bands of 108, 97 and 86 bp, whereas digestion of PCR products of the 577R allele yields bands of 205 and 86 bp. Digested products were then electrophoresed in 5% polyacrylamide gels and silver stained.

### Statistical analysis

Genotype and allele frequencies were compared between elite athletes and controls by the chi-squared test using the statistical package GENEPOL V. 3.4 (updated version of GENEPOL V1.2) [8]. Tests for the HW equilibrium were also done with the same package.

**Table 2** *ACTN3* genotype and allele frequencies in 101 elite Greek track and field athletes and 181 control individuals

Group (n)	Genotype (%)			Allele frequency (%)	
	RR	RX	XX	R	X
*Power athletes (73)	47.94	35.62	16.44 **	65.8	34.2**
Sprinters (34)	73.53	17.65	8.82‡	82.4	17.6‡
Endurance athletes (28)	50	25	25	62.5	37.5
Controls (181)	25.97	55.80	18.23	53.9	46.1

\*\* p < 0.02, ‡ p < 0.001 comparison with controls; \* Power athletes include the 34 sprinters

## Results



A total of 101 Greek elite athletes and 181 control individuals were analysed in this study. ● Table 2 shows the *ACTN3* genotype and allele frequencies from the 73 power-oriented athletes (including 34 sprinters), 28 endurance athletes, and the control group. The observed genotype counts for the power-oriented athlete group as a whole and the control group were not statistically different from those expected under Hardy-Weinberg equilibrium. However, absence of HW equilibrium was found for the small groups of sprinters (extracted from the larger group of power-oriented athletes) and for endurance athletes.

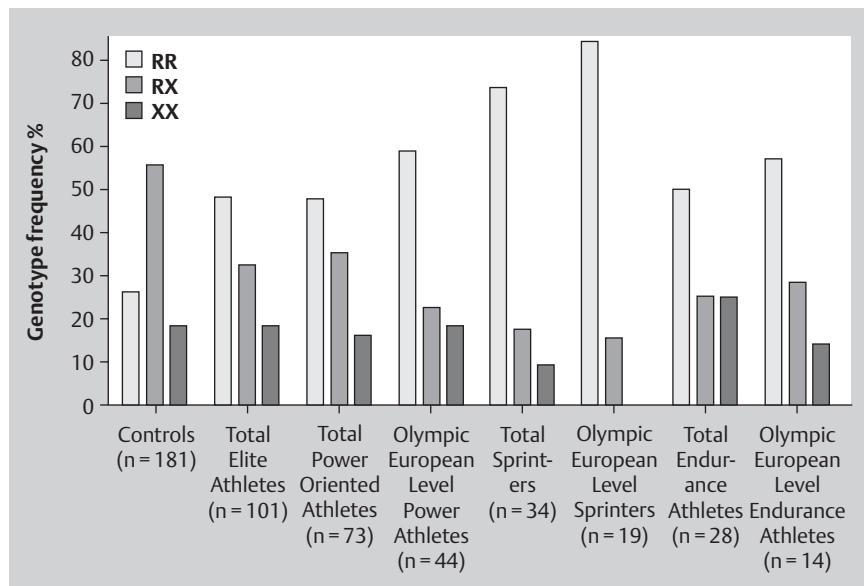
The distribution of the genotypes showed differences among the various groups (● Table 2, Fig. 1). There were statistically significant differences in the frequencies of alleles (p = 0.017) and genotypes (p = 0.016) between elite power-oriented athletes and the control group: the power-oriented athletes displayed a lesser frequency of the X allele than the control group. Statistically significant differences were even more prominent when comparing the allele frequencies (p = 0.0001) and genotype frequencies (p = 0.0001) of the subgroup of sprinters to the control group. It should be noted that all of the Olympic/European-level sprinters in the study sample had at least one R allele for *ACTN3* (● Fig. 1). As compared to the control group, sprinters had a lower frequency of the XX genotype (8.82% vs. 18.23%), and higher frequency of the RR genotype (73.53% vs. 25.97%) (p < 0.0001). In contrast, there was a trend of increased frequency of the XX genotype in the elite endurance athletes group, as compared to sprinters and to the control group (● Table 2). However, there were no significant differences in the frequencies of alleles (p = 0.252) or genotypes (p = 0.238) between the endurance athletes group and the control group or between the power-oriented athletes group and the endurance athletes group for allele (p = 0.742) and genotype (p = 0.771) comparisons.

## Discussion



The current study of elite athletic performance by means of a molecular genetic marker suggests that the presence of  $\alpha$ -actinin-3 protein has a positive effect on power performance, a finding consistent with structural and signalling functions of  $\alpha$ -actinin-3 protein in fast-twitch muscle fibres.

Yang et al. [12] genotyped 107 power-oriented Australian athletes involved in a wide range of sports (including 46 track and field athletes) and found that none of those who had competed at the Olympic level (n = 32) had an XX genotype ( $\alpha$ -actinin-3 deficient). Additionally, in a group of 89 Finnish power-oriented track



**Fig. 1** Frequencies of the *ACTN3* genotypes in 101 elite Greek track and field athletes and 181 control individuals.

and field athletes who competed at the national level, Niemi et al. [6] similarly found that none of these athletes ( $n = 23$ ) had an XX genotype. In our study of a cohort of 73 Greek power-oriented track and field athletes (including 34 sprinters) who competed at the international level, none of the sprinters who had represented Greece in the Olympic Games or in the world or European championships ( $n = 19$ ) had an XX genotype (● Fig. 1). Thus, *ACTN3* is the first skeletal-muscle gene for which such an association has been demonstrated in three different population groups (Australian, Finnish, and Greek) of athletes competing at the Olympic, world, and European level (total sample size:  $32 + 23 + 19 = 69$ ). These findings, in conjunction with the statistically significant differences between the power-oriented athletes groups and controls that were demonstrated in all of the above studies, suggest that the R allele of *ACTN3* gene is an advantageous determinant for achieving high-level sprint-power performance. In Greek sprinters, the X allele of *ACTN3* was notably less frequent and the R allele was more frequent than in endurance runners (● Table 2). This “gene trade-off” was also evident in Australian athletes (power-oriented vs. endurance) [12]. The above data support the hypothesis that the performance constraint, in which decathletes are called upon to perform at high levels in both sprint (100 m) and endurance (1500 m) races [11], may have a genetic basis. These results also show that the frequency of the RR genotype is higher not only when higher-performing athletes are genotyped, but also when a more specific athletic phenotype (running ability) is analysed, suggesting that the phenotypic homogeneity of the study sample (i.e., studying only track and field athletes or sprinters) increases the efficiency of finding candidate genes involved in top human performance. This study demonstrates that sprinting ability is primarily affected by the RR genotype, possibly because sprinting is less influenced by anthropometric and technique factors compared to other sports. In line with this hypothesis and together with the fact that some of the top Olympic/European level athletes (5 high jumpers, 2 decathletes and 1 thrower) in our cohort were XX, it seems possible that success in more complex sports is less influenced by the RR genotype.

A study examining the genotype of East African endurance runners found that although the success of this group is possibly at-

tributable to their genes, surprisingly, only 1% of elite Kenyan runners had an XX *ACTN3* genotype [13]. Our results revealed a trend towards a higher frequency of the XX genotype in the overall group of endurance athletes, but not in the more elite subset of Olympic/European-level endurance athletes (● Fig. 1). The frequency of the RR genotype among 14 Olympic/European-level endurance runners in our study was high (57%) and some of Greek world’s top-level marathon competitors had an RR genotype. This data supports the hypothesis of Lucia et al. [2], that top-level endurance runners require not only slow-twitch myofibres, but also the ability to recruit type II fast-twitch myofibres during the competitively critical phases of an endurance race in which they must effectively sprint for short distances. However, in all studies of endurance Caucasians athletes, the XX genotype predominated: Yang et al. [12] found a higher frequency of the XX genotype in a study of 107 white Australian endurance athletes, as did studies of white professional endurance cyclists of European ancestry [2], and of national-level Finnish endurance athletes [6]. In conclusion, data showing a higher frequency of the XX genotype in our overall endurance-athlete cohort (which included the elite subset of Olympic-level endurance athletes), data from studies of other Caucasian cohorts [2, 6, 12], and the fact that X allele is evolutionary conserved [5, 12] form a basis for the hypothesis that the X allele confers an advantage in endurance performance, although the mechanism by which this occurs is not clear and may involve uncharacterised genetic interactions.

Based on our results and on recent evidence, we suggest the following: It seems that the  $\alpha$ -actinin-3 protein does not have an inhibitory effect in aerobic metabolism, because many Caucasians [2], 99% of Kenyan elite endurance athletes [13], and 75% of Greek international-level competitors in endurance-based sports had at least one R allele of the *ACTN3* gene. The beneficial effect of  $\alpha$ -actinin-3 deficiency on endurance performance is less important than its presence on sprinting-power performance, as four different studies showed that none of the Olympic-level sprinters examined had an  $\alpha$ -actinin-3 deficient XX genotype, but many of the Olympic-level endurance athletes analysed had an RR genotype.

Very little is known about the specific genotypes that contribute to individual responses to training. According to the training principle of individuality, each athlete responds differently to the same quantitative and qualitative training stimuli [10], leading to a range of responses among athletes who have experienced otherwise identical training programmes. The influence of the *ACTN3* R577X polymorphism on each athlete's response to training needs to be more closely examined to explain whether this polymorphism defines the athlete's initial levels of power ability or it actually affects the response to training. Interestingly, Roth et al. [9] recently found that the RR genotype is associated with muscle power response to training and Clarkson et al. [1] showed that XX homozygotes had the lowest baseline strength. Thus, although further investigation is needed to fully understand the function of the *ACTN3* gene, it is evident that *ACTN3* polymorphisms can influence an athlete's power-sprint performance, and we are currently undertaking further genetic studies to investigate this.

The results of this study and of others show that the R allele of *ACTN3* gene is an advantageous allele, in the Olympic-level of sprinting performance, while the X allele may confer some benefit to endurance performance in Caucasians, but is not a determinant for their success in endurance-based sports. Taken together, these results suggest that the *ACTN3* gene can be used as a molecular genetic marker to at least partially predict an athlete's ability to achieve peak power and sprinting performance.

## Acknowledgements



We are grateful to the president of the Greek Olympic Association Niki Mpakogianni and Greek elite athletes whose participation made this work possible. We also thank the director of EUROMEDICA George Chatzinikolaou for his cooperation. The research programme was partially supported by grant number 15/34894 from the Greek Organisation of Football Prognostics.

## References

- Clarkson PM, Devaney JM, Gordish-Dressman H, Thompson PD, Hubal MJ, Urso M, Price TB, Angelopoulos TJ, Gordon PM, Moyna NM, Pescatello LS, Visich PS, Zoeller RF, Seip RL, Hoffman EP. *ACTN3* genotype is associated with increases in muscle strength in response to resistance training in women. *J Appl Physiol* 2005; 99: 154–163
- Lucia A, Gómez-Gallego F, Santiago C, Bandrés F, Earnest G, Rabadán P, Alonso JM, Hoyos J, Córdoba A, Villa G, Foster C. *ACTN3* genotype in professional endurance cyclists. *Int J Sports Med* 2006; 27: 880–884
- MacArthur DG, North KN. A gene for speed? The evolution and function of  $\alpha$ -actinin-3. *BioEssays* 2004; 26: 786–795
- Maniatis T, Fritch EF, Sambrook J. *Molecular Cloning: A Laboratory Manual*. 2nd edn. Cold Spring Harbor, USA: Laboratory Press, 1989
- Mills M, Yang N, Weinberger R, Vander Woude DL, Beggs AH, Easteal S, North K. Differential expression of the actin-binding proteins, alpha-actinin-2 and -3, in different species: implications for the evolution of functional redundancy. *Hum Mol Genet* 2001; 10: 1335–1346
- Niemi AK, Majamaa K. Mitochondrial DNA and *ACTN3* genotypes in Finnish elite endurance and sprint athletes. *Eur J Hum Genet* 2005; 13: 965–969
- Rankinen T, Perusse L, Rauramaa R, Rivera MA, Wolfarth B, Bouchard C. The human gene map for performance and health-related fitness phenotypes: the 2003 update. *Med Sci Sports Exerc* 2004; 36: 1451–1469
- Raymond M, Rousset F. *GENEPOP* (V1.2): population genetics software for exact tests and ecumenicism. *J Heredity* 1995; 86: 248–249
- Roth SM, Delmonico MJ, Rabon-Stith KM, Walsh S, Hurley BF. The *ACTN3* gene R577X polymorphism is associated with muscle power response to strength training *Med Sci Sports Exerc* 2005; 37 (Suppl): S472
- Rushall BS, Pyke FS. *Training for Sports and Fitness*. Melbourne: Macmillan, 1990
- Van Damme R, Wilson RS, Vanhooydonck B, Aerts P. Performance constraints in decathletes. *Nature* 2002; 415: 755–756
- Yang N, MacArthur DG, Gulbin JP, Hahn AG, Beggs AH, Easteal S, North K. *ACTN3* genotype is associated with human elite athletic performance. *Am J Hum Genet* 2003; 73: 627–631
- Yang N, MacArthur D, Wolde B, Onywere VO, Boit MK, Wilson RH, Scott RA, Pitsiladis YP, North K. *ACTN3* genotype is not associated with elite endurance athlete status in Ethiopians and Kenyans. *Med Sci Sports Exerc* 2005; 37 (Suppl): S472