

Streitwolf-Engel *et al.*²² have shown strong differential effects of the fungal species on morphology and pattern of clonal growth of plants, thus affecting their spatial arrangement in communities. Observations such as these and of the present study suggest that mycorrhizae play a significant role in determining the organization of plant communities. Host plants also bring about difference in life-history traits, such as sporulation or infection of different mycorrhizal fungi^{23–25}. Our experiment dealt with a very early phase of seedling growth, and the results of competitive outcome between the host species may change as time progresses. Nevertheless, it throws light on the importance of fungal flora involved in mycorrhizal association to plant community dynamics.

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ACTN3: Athlete gene prevalence in North India

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Alpha actinin is an actin-binding protein involved in anchoring thin filaments of actin to the Z-line of myofibrils. Two structural isoforms of alpha-actinin (ACTN2 and ACTN3) are present in fast twitch (type 2) fibres of the skeletal muscle. ACTN3 gene has two alleles, R and X; the R allele is able to code for full length protein, while no functional protein results from the X allele due to a nonsense mutation (R577X) in exon 16 of ACTN3. The presence of X and R alleles of ACTN3 has been reported to affect the sprinting and endur-

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ance abilities of elite athletes. Therefore, *ACTN3* is also known as the 'athlete gene'. The frequencies of the two alleles show considerable variation in different populations of the world. The aim of the present study was to determine the status of R and X alleles of *ACTN3* in North Indian population. Using PCR-RFLP, we found homozygous R in 27 (22%), heterozygous RX in 76 (61%) and homozygous X in 22 (17%) out of 125 healthy voluntary blood donors. The study revealed that frequencies of both alleles and genotypes of *ACTN3* in North Indians are similar to those of Caucasian populations.

Keywords: *ACTN3*, athlete gene, alpha-actinin, North India.

ALPHA-actinins are an ancient family of actin-binding proteins¹ that play structural and regulatory roles in cytoskeletal organization and muscle contraction. Two skeletal muscle isoforms of alpha-actinin (*ACTN2* and *ACTN3*) are major structural components of the Z-line involved in anchoring actin-containing thin filaments² of the muscle. In humans, *ACTN2* is expressed in all muscle fibres, while *ACTN3* expression is restricted to a subset of fast-twitch type-2 fibres³. Alpha-actinin 3 is the most highly specialized of the four mammalian alpha-actinins, with its expression restricted to fast glycolytic fibres in the skeletal muscle. Alpha-actinin-3 allows fast-twitch fibres to generate greater amount of force at higher velocities of movement^{3,4}. *ACTN3* gene located at 11q13-q14 is responsible^{1,5} for the production of alpha-actinin-3.

Production of *ACTN3* is the result of an individual possessing at least one copy of the R allele⁶. The alternate allele to R is X, which harbours a premature stop codon in exon 16, thus preventing the formation of actinin-3. The difference in the two alleles is that in X a termination codon replaces the amino acid arginine found on the R allele⁷. Presence of the R allele does appear to enhance sprinting ability; while X allele enhances an individual's performance in activities that require a high level of endurance. Several studies have suggested that RR genotype is associated with generating force with high speed. Although a considerable number of individuals in a population are homozygous for this null mutation, there is no disease phenotype associated so far with this deficiency. It is now believed that while *ACTN3* would be desired by a large portion of the population, it is not an essential gene for life⁷. An interesting fact about *ACTN3* is that the percentage of individuals who are homozygous for the X allele varies from population to population. For example, 25% of individuals from the Asian population show homozygous absence of the *ACTN3* gene; for the European population it is 18%, but in African Bantu population⁷, only <1% of the individuals lack a copy of *ACTN3*. We do not have any information about the prevalence of *ACTN3* genotypes in India. Therefore, we undertook the study in our population.

Our study group population was from North India. We included 125 consecutive voluntary blood donors from our blood bank. From each individual, 5 ml blood was collected in EDTA. DNA was extracted from lymphocytes using salting-out method⁸. Polymorphisms were detected by PCR and RFLP (*DdeI*), using forward primer 5'-CTGTTGCCTGTGGTAAGTGGG-3'; reverse 5'-TG-GTCACAG TATGC AGGAGGG-3', corresponding to adjacent intronic sequence. The PCR reaction mixture contained 10 pmol of each primer, 200 μ M of each dNTP (Bangalore Genei, India), 100 ng genomic DNA, and 2 U Taq DNA polymerase (Bangalore Genei). The amplification protocol consisted of initial denaturation step (94°C for 5 min) followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 72°C for 1 min with a final extension of 72°C for 10 min. PCR products were digested at 37°C using 5U of *DdeI* restriction endonuclease (New England Biolab) overnight. Products for *DdeI* digestion were run on 10% polyacrylamide gel and visualized by staining with ethidium bromide (Figure 1). The R and X alleles (codons CGA and TGA respectively) were distinguished by presence (X) or absence (R) of a *DdeI* restriction site in the PCR digestion products. Direct counting method was used to determine the frequency of genotypes and alleles. Chi-square test was used to determine Hardy-Weinberg equilibrium.

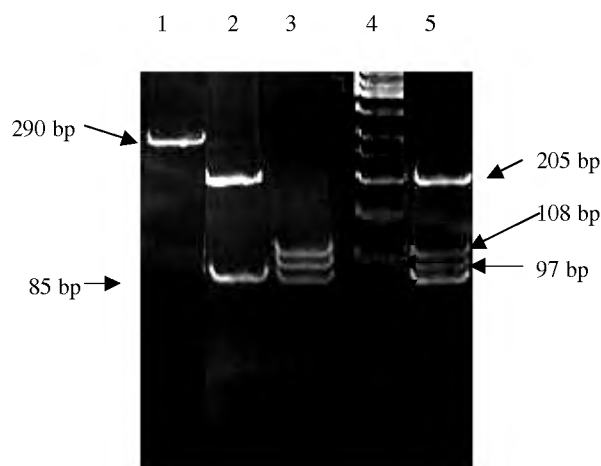


Figure 1. PCR-RFLP genotyping of *ACTN3* on 10% PAGE. Lane 1, Undigested PCR product 290 bp; lane 2, RR genotype; lane 3, XX genotype; lane 4, 50 bp ladder and lane 5, RX genotype.

Table 1. Genotype and allele frequencies of *ACTN3* in North Indians

Genotype/allele	Total (n = 125)	Frequency
RR	27	0.22
RX	76	0.61
XX	22	0.17
R	130*	0.52
X	120*	0.48

*Number of alleles = 250.

Table 2. Comparison of genotype and allele frequencies of *ACTN3* in different populations

Ethnic group ¹	No. of chromosomes	Frequency of genotype			Allele frequency 577X
		RR	RX	XX	
Asian	56	0.25	0.50	0.25	0.5
Javanese	96	0.17	0.58	0.25	0.54
European White	214	0.36	0.44	0.20	0.42
Hispanic	64	0.34	0.50	0.16	0.41
Aboriginal Australian	174	0.52	0.38	0.10	0.29
African American	90	0.6	0.27	0.13	0.27
African Bantu	156	0.81	0.18	0.01	0.1
North Indian	250	0.22	0.61	0.17	0.48

Distribution of *ACTN3* *DdeI* genotype was RR 22%, RX 61%, XX 17% and allelic distributions were 52 and 48% respectively, for R and X (Table 1). All alleles and genotypes were in Hardy-Weinberg equilibrium (Table 1). Mills *et al.*¹ reported the prevalence of *ACTN3* mutation in four major human groups (Asia/Americas, Australasia, Africa and Europe). The X allele was more frequent in Eurasia (0.51) and least in Africa (0.16). Frequency of the X allele in the African Bantu samples was significantly lower than that of all other populations. Yang and co-workers⁷ investigated the frequency of the mutation in a cohort of 429 international-class athletes representing 14 different sports and 436 unrelated controls. In 107 elite sprint and power athletes, they found a significantly lower frequency of the XX genotype compared to the controls.

Each of the alleles conveys to its host a particular advantage⁹⁻¹¹. While the R allele does appear to enhance sprinting ability, the X allele enhances an individual's performance in activities that require a high level of endurance. Since most humans require a combination of the two abilities, it would seem that we would be selected to possess a copy of each allele.

Performance in sports has long been attributed to genetic make-up of an individual. As shown in this study in the North Indian population, frequencies of both alleles and genotypes are similar to those of the Caucasian population. Thus the vast differences in athletic performance cannot be explained solely on the basis of *ACTN3* genotypes. There is still possibility of different genetic make-up in terms of other genes. In addition, various environmental factors, including nutrition and socio-economic conditions, significantly affect the athletic abilities of individuals.

In India, there is a heterogeneous population and it would be desirable to find out the gene frequency in sub-populations of the country. Although the genetic test is being promoted for choosing an appropriate sport career, the *ACTN3* test does not guarantee a person's ability to be an elite athlete. In future, a battery of genetic tests may increasingly play a role in identifying the biological potential of the child.

In conclusion, the North Indian population frequency of R allele of *ACTN3* is 0.52 and that of X allele is 0.48.

The frequency of homozygous R is 0.22 and that of homozygous X is 0.17; heterozygosity was 0.61.

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