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Original Article

In the search of a ‘fitness gene’: an analysis of ACTN gene polymorphisms in serving soldiers

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ABSTRACT

Background: Genetic polymorphisms in the exon 15 and exon 16 of the ACTN3 gene are believed to be associated with athletic performance. Paratroopers are some of the fittest soldiers in the Indian Armed Forces. This study was taken up to assess if there was a significant difference in the genetic profile between paratroopers and non-paratroopers.

Method: Polymerase chain reaction (PCR) followed by restriction length fragment polymorphism (RFLP) was used to analyse the genetic polymorphisms in the exon 15 and 16 of the ACTN3 gene.

Results: There was a significant difference between paratroopers and non-paratroopers in the polymorphic loci at codon 15 and 16.

Conclusions: The study suggests that there is a significant difference in the genotype between paratroopers and non-paratroopers. It is likely that the differences in muscle fibres as a result of these genotypic changes confer a ‘survival advantage’; people with a homozygous genotype are more likely to pass the harsh probation and qualify for the Parachute Regiment.

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Introduction

The α -Actinins are a family of actin-binding proteins related to dystrophin and the spectrins.¹ In skeletal muscle, α -actinin 2 and α -actinin 3 are the major components of sarcomeric Z lines where they anchor the actin-containing thin filaments in a

constitutive manner.² Sarcomeric α -actinins also bind to other proteins like nebulin, myotelin and myozenin and are important in playing a role in thin filament organisation and the interaction between the sarcomeric cytoskeleton and the cell membrane.^{3–5}

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Two skeletal muscle isoforms of alpha-actinin (ACTN2 and ACTN3) are present. In humans, ACTN2 is expressed in all muscle fibres, while ACTN3 expression is restricted to a subset of fast-twitch type-2 fibres.⁶ ACTN3 is the most highly specialised of the four mammalian α -actinins, with its expression restricted to fast glycolytic fibres in the skeletal muscle. ACTN3 allows fast-twitch fibres to generate greater amount of force at higher velocities of movement.^{2,6} ACTN3 gene located at 11q13-q14 is responsible for the production of α -actinin 3.^{7,8}

Gene polymorphisms are normal variants in the human genome. DNA sequences of the human genome reveal that many genes are polymorphic. In the coding or noncoding regions of specific genes, there may be either a single base pair substitution or a variable number of repeats of a short repetitive DNA sequence (VNTR). These variations or polymorphisms may influence the rate of gene transcription, the stability of the messenger RNA or the quantity and activity of the resulting protein.

Two independent polymorphisms in exons 15 and 16 of the ACTN3 gene are proposed functional variants with possible influence on athletic performance. In exon 15, an A $\rightarrow$ G transition mutation occurs, which changes the nucleotide codon sequence from CAG to CGG. The CAG codon that once coded for glutamine (Q) now codes for arginine (R). This nucleotide alteration introduces a cleavage recognition site for the restriction enzyme *MspI* in the 523R allele. In exon 16, a nonsense point mutation (C $\rightarrow$ T) alters a codon in the exon sequence from CGA to TGA (rs 1815739). This changes the amino acid from an arginine (R) to a premature stop codon (X).

Production of the complete ACTN3 is the result of an individual possessing at least one copy of the R allele.⁹ 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 allele.¹⁰ The presence of the X allele helps in 'endurance activities', while the R genotype helps an individual in generating force with high speed. Although the X allele of the ACTN3 gene would be desired by a large number of people, an absence of the protein or the presence of a truncated protein is not associated with any deleterious effects.

Elite athletic performance is a complex trait, which displays a genetic trade-off between speed and endurance.¹⁰ The 577XX genotype of the ACTN3 gene leads to a loss of α -actinin 3 in fast-twitch muscle fibres and is more common among endurance athletes, whereas the wild-type 577RR genotype is more common among sprinters.¹⁰

Although studies evaluating the genetic polymorphisms in the exon 16 of the ACTN gene are available, the authors did not come across any studies evaluating the exon 15 polymorphisms of the ACTN gene in endurance and sprint athletes. In a way, this would then be a pilot study where these polymorphisms would be evaluated.

Paratroopers are considered to be elite forces of any army. The physical fitness of the paratroopers is considered to be more than that of the other fighting forces. It is true that physical fitness is dependant considerably on the grade and level of training. However, it is not clear if genetic influences also play a part. Do genetic polymorphisms influence the level of physical fitness in paratroopers who are at their peak of

physical performance? Alternatively, is it that the grade of training influences the physical fitness and genetic influences do not play any role in the fitness?

This cross-sectional study was taken up to evaluate if there is a significant difference in the polymorphisms in the ACTN3 gene between paratroopers and non-paratroopers. Paratroopers are made to undergo probation before they are finally selected for the Parachute Regiment. The probation grades the degree of physical fitness and determines if the soldier would be suitable for the Parachute Regiment. An evaluation of the genetic polymorphisms would determine if the high level of physical fitness achieved by the soldiers of the Parachute Regiment is entirely based on the grade and level of training or of there is a certain degree of genetic influence as well.

Materials and methods

A total of 137 soldiers in this study, 67 paratroopers and 70 non-paratroopers were recruited for this study. Age was not an exclusion criteria, since only the genetic polymorphisms were being evaluated and the study did not include an evaluation of physical fitness. Similarly, the geographical origin of the soldiers was not used as an exclusion or inclusion criteria.

Genomic DNA was extracted from the ethylenediaminetetraacetic acid-whole blood using QIAGEN DNA Mini kit. Genotyping of the exon 15 and 16 of the ACTN gene was performed using the polymerase chain reaction (PCR), followed by restriction length fragment polymorphism (RFLP) analysis.¹¹ The primer sequences are outlined in Table 1. PCR was performed as described by earlier authors.¹¹ The product size is shown in Table 1. The exon 15- and exon 16-amplified fragments were digested by *MspI* and *DdeI*, respectively, in conditions recommended by the supplier. The digested products were then electrophoresed on a 3% agarose gel for identifying the genotype.

In case of the exon 15 polymorphism, there is a restriction cut site for the enzyme *MspI* in the 523R allele. Digestion of the 192-bp PCR product cleaves the 523Q allele into two fragments of 118 and 74 bp, respectively. Digestion of the 523R allele generates fragments of 97, 74 and 21 bp. RFLP of exon 16 was done as described by earlier authors.¹¹ A gel picture of the RFLP analysis of exon 16 is shown in Fig. 1.

Table 1 – Primer sequences and the size of the product generated after PCR.

Primer	Primer sequence	Size of product
Exon 15 forward	GGTGGGTAGGTGGGTGAGGC	192 bp
Exon 15 reverse	GAGTGTAACAGCCACACGTCC	
Exon 16 forward	CTGTTGCCTGTGGTAAGTGGG	291 bp
Exon 16 reverse	TGGTCACAGTATGCAGGAGGG	
PCR, polymerase chain reaction.

Lane 1 and Lane 20 - 100 bp ladder

Lane 2, 5, 10, 14, 16 - RR Genotype. 205 and 85 bp

Lane 3, 6, 7, 8, 11, 17, 19 - XX Genotype. 108, 97 and 85 bp.

Lane 4, 9, 12, 13, 15, 18 - RX Genotype. 205, 108, 97 and 85 bp

Fig. 1 – Gel picture showing the different alleles of exon 16 after digestion of the PCR product. PCR, polymerase chain reaction.

Statistical analysis

Statistical analysis was performed using the SPSS test for Windows (version 13). The Pearson's correlation coefficient was used to calculate the correlations between the different variables. A *p* value of 0.05 was considered statistically significant.

Results

Demographic profile

A total of 137 soldiers were evaluated. 67 paratroopers and 70 non-paratroopers were considered in the study. Paratroopers were recruited from the Special Forces, and non-paratroopers were recruited from the army as well as the services. The age of the paratroopers ranged from 24 years to 45 years with an average of 32.95 years. The age of the non-paratroopers ranged from 21 years to 30 years with an average of 22.5 years. No specific geographical belt was specified when the subjects were recruited.

Body mass index of the groups

The average body mass index (BMI) of the paratroopers was 22.42, and the average BMI of the non-paratroopers was 21.11. Since there was no significant difference between the BMI of the two groups, no further analysis was carried out regarding the BMI and genetic polymorphisms.

Codon 15 polymorphisms in relations to the groups

When the codon 15 polymorphisms were analysed in relation to the groups, it was seen that the QR genotype was seen more commonly in non-paratroopers (52.86%) as opposed to paratroopers (29.85%). The RR genotype was seen less commonly in non-paratroopers (35.71%) as compared to paratroopers (43.28%), and the QQ genotype was seen less commonly in non-paratroopers (11.43%) as opposed to paratroopers (26.86%). The codon 15 polymorphism was strongly correlated with the genotype (*p* = 0.010). The results are shown in Table 2.

Codon 16 polymorphisms in relations to the groups

When the codon 16 polymorphisms were analysed in relation to the groups, it was seen that the RX genotype was seen more

Table 2 – Codon 15 polymorphisms in relationship to the two groups.

Groups	RR	QR	QQ	Total
Nonparatroopers	25 (35.71)	37 (52.86)	8 (11.43)	70 (100)
Paratroopers	29 (43.28)	20 (29.85)	18 (26.86)	67 (100)
Total	54	57	26	137
<i>p</i> = 0.010				

Figures in brackets denote the percentages.

Table 3 – Codon 16 polymorphisms in relationship to the two groups.

Groups	RR	RX	XX	Total
NonParatroopers	14 (20.00)	41 (58.57)	15 (21.43)	70 (100)
Paratroopers	21 (31.34)	20 (29.85)	26 (38.81)	67 (100)
Total	35	61	41	137
<i>p</i> = 0.004				

Figures in brackets denote the percentages.

Table 4 – Frequencies of the alleles in codon 16 polymorphisms in different ethnic groups.

S No	Ethnic population	RR	RX	XX	Reference
1	North Indians	22%	61%	17%	11
2	Indian athletes	19%	46%	54%	12
3	Indian control population	16%	45%	39%	12
4	Finnish endurance athletes	50%	38.3%	11.66%	13
5	Finnish sprinters	50.5%	42.85%	6.6%	13
6	Chinese soldiers	39.8%	43.4%	16.8%	14
7	Present study – non-paratroopers	20%	58.57%	21.43%	—
8	Present study – paratroopers	31.34%	29.85%	38.81%	—

commonly in non-paratroopers (58.57%) than in paratroopers (29.85%). The RR genotype was seen less commonly in non-paratroopers (20.00%) as compared to paratroopers (31.34%). The XX genotype was seen less commonly in non-paratroopers (21.43%) as opposed to paratroopers (38.81%). The RR and the XX genotypes were more common in paratroopers. There was a significant difference between the haplotypes of the codon 16 polymorphism of the two groups ($p = 0.004$). The results are shown in [Table 3](#).

Discussion

In the present study, we found that the frequencies of the RR and XX polymorphisms in non-paratroopers were similar to the literature available worldwide. [Table 4](#) lists the frequencies of the genotypes which have been seen in different studies.

In the present study, we also found that the RR and the XX genotypes of codon 16 polymorphisms were found more commonly in paratroopers when compared to non-paratroopers. The RR genotype is associated with the presence of α -actinin 3 in fast-twitch muscle fibres and is more common among sprinters. The XX genotype is associated with a loss of α -actinin 3 in fast-twitch muscle fibres and is usually seen in endurance athletes.^{[12](#)}

In the present study, we found that paratroopers had both the genotypes, of sprinters and endurance runners. The RX genotype was not commonly seen. Both endurance and sprinting are required for these highly physically fit individuals. We did not statistically analyse if the genotypes were associated with speed or endurance tests. However, our study suggests that paratroopers usually have the RR or the XX genotype which probably gives them a selection advantage during the probation period since both speed and endurance tests are a part of the probation training. The RX genotype which does not confer an advantage to either sprint or endurance training is under-represented in the paratroopers, suggesting that this heterozygous genotype does not confer any advantage during the probation training.

In a study evaluating the ACTN3 R577X polymorphism and performance phenotypes in young Chinese male soldiers, Shang et al. compared the genotypes of Chinese soldiers and young Chinese males.^{[14](#)} They did not find any correlation between the genotypes and the sprint/endurance phenotypes. This is not in agreement with the present study. This is perhaps because the present study has chosen two very different populations (paratroopers and non-paratroopers)

and compared the genotypes as against the above-quoted study where the level of physical fitness of soldiers was not specified.

We did not find any literature suggesting that the R/Q genetic polymorphism at codon 15 is a functional one. Polymorphisms in exon 15 have not been reported in relation to athletic performance. In our study, we found that the RR and the QQ genotypes of the codon 15 gene polymorphisms were over-represented in the paratroopers as compared to the non-paratroopers. The exon 15 polymorphism results in allelic variants distinguished by encoding either an arginine or glutamine at amino acid residue 523 in the full-length protein. While arginine is positively charged, and glutamine is uncharged, both of these amino acids carry polar side chains. It is possible that this substitution significantly alters the structure or function of the protein. Alternatively, the exon 15 polymorphism may only present an effect in conjunction with other genetic variations.

In conclusion, the study suggests that there is a significant difference in the genotype between paratroopers and non-paratroopers. We really do not know how important this difference in genotype is related to athletic performance. Many different genetic and environmental factors are related to athletic performance, and these genetic polymorphisms are only two such factors. It has been estimated that the R577X polymorphism accounts for only 2.2% in the baseline muscle strength in females.^{[15](#)} Therefore, the genetic polymorphisms of the ACTN gene are only one of a myriad of complex, interacting factors that influence muscle performance. However, because of the statistically significant genotypic differences between paratroopers and non-paratroopers, we can still speculate that the genotype also helps in the selection of this subset of soldiers and also helps them maintain their rigorous physical fitness.

Conflicts of interest

The authors have none to declare.

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