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ACTN3 R577X Polymorphism in Asian Indian Athletes

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ABSTRACT Replacement of Arginine (R) by a premature stop codon (X) (R577X, rs1815739) in Alpha-actinin 3 gene (*ACTN3*) has been commonly reported. The R allele has been hypothesized to be associated with power-based performance, while the X allele may be linked with endurance abilities. To evaluate if this theory holds true in Indian subjects, we studied the ACTN3 genotype distribution in 305 Asian Indians, comprising of 155 athletes and 150 normal sedentary individuals. We did not observe any significant difference in the allelic frequencies between athletes *(R/X*=0.42/0.58) and normal subjects (*(R/X*=0.39/0.61). But, while categorizing the athletes on the basis of their competitive level (National/ International vs. Regional); the genotype distribution pattern became more prominent (χ^2 = 4.9, d.f=2, p=0.08), though not statistically significant. Additionally, in the National/International athletes, R allele was seen to be associated with power based performance (χ^2 = 6.6, d.f=1, p<0.01). In the same group, a high frequency of X allele was observed with endurance performance (χ^2 = 3.14, d.f=1, p=0.07), but fell short of significance. Thus, XX genotype could be an important factor in determining endurance performance. However, the study needs to be expanded to a larger cohort of National/ International athletes. To our information, this is the first Indian study on *ACTN3* genotype and its association with sporting performance.

INTRODUCTION

Alpha actinins are a highly conserved family of actin binding proteins belonging to the spectrin protein superfamily. These proteins play an important role in structural and regulatory functions in cytoskeletal organization and muscle contraction. There are four genes so far reported for α-actinin in humans: ACTN1, ACTN2, ACTN3, and ACTN4. The ACTN1 and ACTN4 code for non-muscle proteins, whereas ACTN2 and ACTN3 code for myofibrillar proteins localized at the Z disk (Clarkson et al. 2005). Two sarcomeric α-actinin isoforms exist in humans, α-actinin-2 and α-actinin-3. While α -actinin-2 is expressed in all types of muscle fibers, the expression of α-actinin-3 is almost exclusively restricted to fast, glycolytic type II fibers. They constitute the predominant protein component of the sarcomeric Z line, where they form a lattice structure that anchors together the actin containing thin filaments and stabilizes the muscle contractile apparatus (MacArthur and North 2004).

Alpha actinin-3 is expressed by the *ACTN3* gene, located on the long arm of chromosome 11q13.2. Alpha-actinin-3 allows fast-twitch fibers to generate greater amount of force at higher velocities of movement. In 1999, a functional Single Nucleotide Polymorphism (SNP) was identified in the *ACTN3* gene (C>T transition at nucleotide 1747 in exon 16); where Arginine (R) at amino acid position 577 is replaced by a premature stop codon (X). This SNP R577X (rs.1815739) leads to the deficiency or absence of *ACTN3* protein (North 1999).

Research studies carried out in mouse models have provided the exact mechanism by which deficiency of *ACTN3* leads to the shift from power based performance to endurance excellence. *ACTN3* knock-out mice have been observed to present with reduced fast fiber diameter, increased activity of multiple enzymes in the aerobic and more energy efficient metabolic pathways, enhanced recovery from fatigue, all suggesting the characteristics of slow fibers (MacArthur et al. 2008). *ACTN3* gene is also known as the 'athlete gene' since X and R alleles are reported to affect the sprinting and endurance abilities of elite athlete.

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Numerous studies have been conducted concerning the determination of association of the alpha actinin-3 gene *(ACTN3)* polymorphism with human physical performance and elite athlete status. The 577R allele and the RR genotype of the *ACTN3* R577X polymorphism have been found to be associated with top-level sprinters in a broad variety of ethnic groups (Yang et al. 2003; Druzhevskaya et al. 2008). Other study groups have failed to find an association of the 577X genotype variation with endurance athletes in Spain (Lucia et al. 2006; Lucia et al. 2007) and East and West Africa (Yang et al. 2007). However, to the best of our knowledge, no study has evaluated the *ACTN3* R577X polymorphism in Indian athletes.

The aim of this study was to analyze the allelic and genotypic frequency of *ACTN3* gene in selected Asian Indian population, and to subsequently evaluate if *ACTN3* R577X polymorphism was associated with the sporting ability in National/International level athletes.

MATERIAL AND METHODS

Subjects

Three hundred and five subjects of Asian Indian origin were enrolled in the present study. Of these, 155 subjects were athletes (111 men and 44 women, age between 14 and 40 years) who volunteered to participate in this study. The control group comprised of 150 non-athletic healthy individuals from the similar population group. All the participants, including controls were of Indo-European ethnicity and written informed consent was obtained from each volunteer of study and control group.

The athletes were categorized by their competitive levels, viz. Regional and National/International. Of the 155 athletes, 72 were top-level athletes of the country, representing India at National and International sporting events, while 83 were Regional (Varsity to State level) athletes, all pursuing their sport for more than 4 years.

The top level athletes were subdivided by their sporting excellence, viz. Power Sports (for example, running <200m, swimming 50m-100m, artistic gymnastics), Mixed Pattern sports (for example, basketball, tennis, volleyball) and Endurance Sports (for example, running > 800m, swimming > 400m, hockey).

Genotyping Method

Genomic DNA was extracted from the EDTA-Whole blood using QIAGEN DNA Mini kit. Genotyping of the *ACTN3* R577X polymorphism was performed using the Polymerase Chain Reaction, followed by Restriction Length Fragment Polymorphism (RFLP) analysis (Goel and Mittal 2007). In brief, the method comprised of amplification of the *ACTN3* gene using the primers *ACTN3*-F: 5'-CTGTTGCCTGTGGTAAGTGGG-3' and *ACTN3*-R: 5'- TGGTCACAGTATGCAGGAGGG-3'. PCR was performed by initial denaturation at 95°C for 3 minutes, followed by 35 cycles of denaturation at 94°C for 30s, annealing at 53 °C for 45s, extension at 72 ° C for 45s, and a final extension step of 5 min at 72°C. The amplified fragment was digested by DdeI (New England Biolabs, Beverly, MA) in a condition recommended by the supplier. The digested products were then electrophoresed on a 2.5 % agarose gel for identifying the genotype. Genotypes were confirmed by DNA sequencing.

Data Analysis

Allelic frequencies were determined by direct counting. A χ^2 test using the GraphPad In Stat Statistical Package was used to confirm that the observed genotype frequencies and were found to be in Hardy-Weinberg equilibrium. Genotype frequencies at different competitive levels were also compared. The allelic frequencies amongst National/International level athletes excelling in a specific sporting type (power/endurance/mixed pattern) were compared to the total allelic frequencies in these athletes.

RESULTS

Three genotypes of the *ACTN3* gene were identified on the basis of the RFLP pattern, as illustrated in Figure 1. The complete data on the *ACTN3* R577X genotype and allelic frequencies is illustrated in Table 1, which was in agreement with Hardy-Weinberg equilibrium. We compared the two sub-groups of Regional and National level athletes for the genotype frequency, as illustrated in Table 2. Interestingly, the genotype distribution pattern became more prominent ($\chi^2 = 4.9$, d.f=2, p=0.08), though not statistically significant.

Further, we evaluated the sub-group of National/International level athletes to identify if

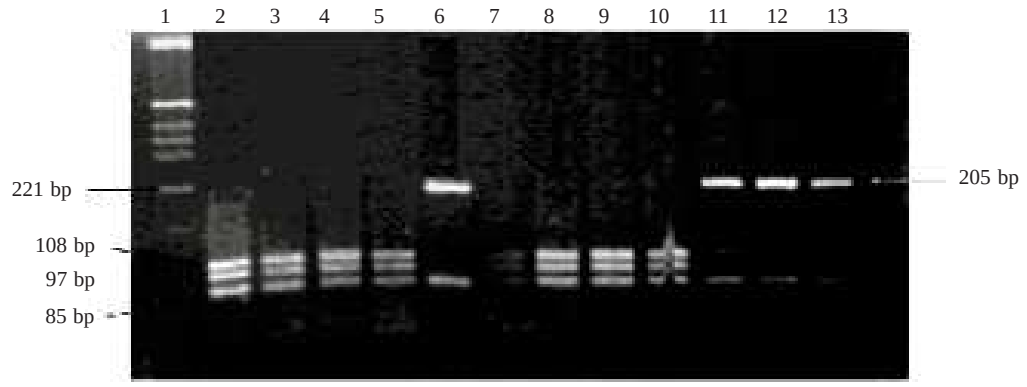

Fig. 1. ACTN3 Genotyping inathletes – PCR-RFLP (DdeI)
Lane 1: **pBR322 Hinf I Molecular weight marker, Lanes 2-5 and 7-10: XX genotype, Lanes 6 and 12: RR genotype, Lanes 11 and 13: RX genotype**

Table 1: Genotype and allelic frequencies of *ACTN3* R577X in controls and athletes

ACTN3 R577X	*N*	*Genotype*			χ^2	*p*	*Allele frequencies*		χ^2	*p*
		RR	*RX*	*XX*			*R allele*	*X allele*		
Athletes	155	30 (19)	71 (46)	54 (35)	3.5*	0.17	131 (42)	179 (58)	0.81#	0.36
Controls	150	24 (16)	68 (45)	58 (39)			116 (39)	184 (61)		

Data in parentheses indicate relative values; *d.f = 2 ; #d.f = 1

the hypothesis of R577X association with power sports held true in Indian athletes. R allele was observed at a frequency of 71%, and was associated with the power sporting ability ($\chi^2 = 6.6$, p =0.01), when compared to the R allele frequency in all National/International level athletes. In such power athletes, X allele was underrepresented (29%). On the contrary, X allele was represented higher (69%) as compared to the R allele (31%) in endurance athletes; however the association of X allele with endurance athletes was not significant (p>0.05). The allelic frequency in the mixed sport type was identical to the controls, showing no preference for any particular allele.

DISCUSSION

The current study corroborates the earlier findings that the *ACTN3* R allele is strongly associated with the power sporting ability in top-level athletes that represent a country at National or International level. However, *ACTN3* X allele is not significantly associated with the endurance sporting ability. Association of *ACTN3* R577X polymorphism was first hypothesized and illustrated by Yang et al. (2003), who reported the frequency of R allele at 72% in power sportspersons (sprinters). The group from North India had reported frequency of R and X alleles and genotypes of *ACTN3* was similar to that of Caucasian population (Goel and Mittal 2007). They had studied the distribution of *ACTN3* genotypes in healthy blood donors and have compared various ethnic groups. However, the aim of the present study is to find out the association of *ACTN3* genotypes in athletes and non-athletes. The genotype frequency reported by Goel and Mittal (2007) was RR 22%, RX 61%, XX 17%, which is slightly different from our

Table 2: Genotype frequencies of *ACTN3* R577X in 155 athletes at different competitive levels

ACTN3R577X	*N*	*Genotype*			χ^2	*p*
		RR	*RX*	*XX*		
Regional athletes	83	14 (17)	44 (53)	25 (30)	4.9	0.08
National athletes	72	14 (20)	29 (40)	29 (40)		

Data in parentheses indicate relative values; d.f. = 2

study (Table 1) while the frequency of RX genotype is predominant in both these studies based on Indian population.

We started with a cohort of 155 athletes, all playing their respective sports for no less than 4 years. When compared with the controls, no significant difference in the *ACTN3* genotype frequencies was observed. However, when the genotype frequencies between Regional level and National level athletes were compared, the distribution pattern became more prominent.

We evaluated 72 top-level athletes representing India on a National/International level, segregated on their sporting excellence where we observed significant association of the *ACTN3* R allele (71%) with power-based sports. Consistent with this finding, association of *ACTN3* R allele with the elite athletic performance has been demonstrated in Finnish (Niemi and Majamaa 2005), Israeli (Eynon et al. 2009) and Russian (Druzhevskaya et al. 2008) athletes.

We presume that the evidence of association of *ACTN3* R577X polymorphism in top-level athletes competing at national and international levels, as compared to the absence of such an association in regional athletes, may be partly due to the fact that the national/international athletes were observed to have channelized sporting participation and excellence, while regional athletes were participants of multiple sporting types (track and field as well as ball games) at Varsity to State level.

Secondly, the sporting excellence was varied as observed by their competitive level (Regional Vs. National/International). It is possible that identification of the *ACTN3* genotype may aid some of these regional athletes (especially Varsity level) to modify their training regime. However, there are contradictory reports (Lucia et al. 2007) who reported a case of a Spanish elite power athlete bearing an XX genotype. So the association of *ACTN3* R allele or RR genotype in power sports definitely supports, but is not an absolute critical factor in the development of an elite power sportsperson.

Another noteworthy observation in our study was that the X allele or the XX genotype was present at higher frequency in endurance athletes (69%), but the association fell short of significance (p=0.07). Enyon et al. (2009) have reported that endurance performance seems to be multi-factorial and an oligogenic trait, where two or more genes are presumed to be acting in tandem. Additionally, Lucia et al. (2006) in their cohort of male cyclists have demonstrated that the XX genotype does not confer any advantage to their extreme endurance performance and that *ACTN3* genotypes do not appear to influence human extreme endurance performance like cycling .

The XX genotype distribution in our control group (39%) was higher than the studies of Israeli cohort (18%) (Eynon et al. 2009), Russian cohort (14.2%) (Druzhevskaya et al. 2008) and the Australian cohort (18%) (Yang et al. 2007). This variation in frequency may be attributed to the idea that 577X allele may be population specific. However, McArthur and North (2004) have suggested that the 577X allele might have increased in some populations due to natural selection. Reports also suggest that a region of genomic DNA around the human R577X allele carries a signature of this selection, suggesting that the loss of α-actinin-3 expression was advantageous during the adaptation of modern humans to the Eurasian environment (MacArthur et al. 2008).

Of the 155 athletes, we had 44 female athletes. Here, we observed that none of the power athletes (0/3) had an XX genotype. Though this is in agreement with the findings of Yang et al. (2003), it is too small a number to arrive at any association in our cohort. We also observed a higher representation of the XX genotype (12/21) in the female endurance athletes.

CONCLUSION

Our data indicated a positive association for *ACTN3* 577R allele with power sports at National/International level athletic performance; while the 577X allele is not critical but aids in the endurance performance. No significant difference was found in the allelic frequencies between the athletes and normal subjects. The study emphasizes the need for carrying out a larger cohort study for better understanding of the association, if any of *ACTN3* genotype variants and sporting excellence. Such studies may further help in channelizing the sporting abilities of budding athletes, when used with more generic factors like nutrition, training regimes, and coaching.

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