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A molecular insight in sport and athletic alpha – *actin3* gene in students of Physical Education Faculty and bodybuilders

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ABSTRACT

Alpha – *actin3* is gene for athletic performance and sport. Three types of genotypes were identified within the gene: RR homozygous, RX heterozygous, and XX which lacks expression of these fibers. All three genotypes were identified among participants. Distribution of these genotypes was significant them, and it is noticeable that XX genotype was less in numbers than the other genotypes. BMI and BFP were in acceptable limits in athletes, but it was found to increase in control group with age. Two males and one female exhibited odd reading regarding muscle mass and testosterone level which was significantly higher than the others. Genetic analysis of these athletes showed SNPs that altered ORFs reading site, and production of different protein from the gene which affected muscle mass dramatically toward higher density, and triggered higher testosterone level in the body as an exceled response to intensive training. The three of them were found to be of XX genotype. Among athletic participants, physical fitness was high especially with young athletes. Most sprinters were found to be RR and RX genotype, while endurance athletes were of XX genotype. Significant SNPs at *actin3* gene determined exceled athletes who were found to comprise less than 1%.

Keywords: *actin3* gene, genotyping, exceled sport performance, muscle fibers composition

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1. Introduction

The family of α-*actin* comprises structural components of the Z-membrane of the muscle, since they form a link between the thin actin fibers and stabilize the muscle contracting mesh [1]. Expression product of α-*actin3* (ACTN3) is limited to fast muscle fibers responsible for functioning to generate momentum when speeding. About 16% worldwide population was estimated to show congenital deficiency of alpha-*actin3* based on a common nonsense polymorphism in the ACTN3 gene with no disease phenotype associated with this deficiency which might conclude that other factors would neutralize the dysfunction of alpha-*actin3* where skeletal muscle of fast Z lines are found [2]. The (RS1815739) in the majority of people in many ethnic groups comes with 0.22 +/- 0.05 to 0.52 +/- 0.04 allele frequency [3].

Both genders of elite athletes had significantly more frequencies of the 577R allele compared with controls, which suggested that the presence of alpha-*actin3* has a benefit influence on the function of skeletal muscle in producing force and power at high-speed sprinting, and give developmental advantage because of increased sprint performance.

ACTN3 genotype is related to speed and power phenotypes [4]. Estimated that elite sprinting athletes had significantly more frequencies of the RR genotype, a result that has been reported frequently to explain speed, power and strength athletes [5] [6] [7] [8] [9] [10]. Nevertheless, it was reported an increased XX genotype frequency in endurance athletes vs. controls, this relationship is less reliable, with most studies reporting a lack of association between XX genotype and endurance performance [11].

Genetic analysis studies were performed to influence the performance of athletes. Genes give reliable information about many characteristics of the body. Genome-wide linkage analysis represents a powerful mean to detect sites where genes influence performance characteristics. This technique includes examining genetic

markers spread over the human genome and researching the relationship between each marker and its specific phenotype features [12].
When the genetic trends are considered, it is seen that the age, gender, anatomical criteria, nervous response, psychological fitness as well as biological motor skills and cardiovascular function of the athlete are the determinants of their athletic performance [13].
It is notable that in a cold-water immersion challenge experiment, humans lacking alpha-*actin3* (XX homozygotes) were superior in maintaining core body temperature due to changes in skeletal muscle thermogenesis [14].
This study was designed to determine the following parameters: first investigating the precise location of RS1815739 location on *actin3 3* gene, molecular impact of specific SNPs on gene product regarding protein difference which eventually affects muscle structure in exceled athletes, distribution and significance of such SNPs on gene function, molecular insight associated with *actin3* gene.

2. Materials and methods

2.1. Study approval
This study was approved by Al-Esraa University College IRB and Ethical committee under reference No. 2337.

2.2. Participants consent
Athletes from both genders (200 male divided as 150 sprinter, 50 power lifter and endurance athletes, 150 female divided as 120 sprinter and 30 endurance athletes) from College of Physical Education with age ranged from 18 – 30 year old. Control group comprised of 75 persons (50 male and 25 female non athletic individuals) with the same age groups of college students. All participants were asked to give their written consent. In addition, 170 participants from bodybuilding gyms were included in the study distributed as follow, 70 bodybuilder from PowerFitness Gym, 30 bodybuilder from Black Dragon Gym at Al-Karrada district, 40 bodybuilder from Oxygen Gym and 30 from Platinum Gym at Al-Mansour District in city of Baghdad. Age distribution for bodybuilders ranged from 20 to 50 years old with at least 2 year of experience in bodybuilding. Control group for bodybuilder included 100 participants with the same age category. Athletes under age 18 years were not allowed to participate in the study since they are under age. Personal data and private information were kept unrevealed and participants were provided with their genotype after analysis coping with their request confidentially.

2.3. Samples and specimens
Samples in this study were mainly blood from puncture wound collected aseptically at site. A 5 ml of blood was kept fresh in collection tubes under cooling conditions and transferred immediately to the laboratory for further processing.

2.4. Measurement of testosterone level
Serum testosterone was measured in all athletes from College of Physical Education using I- chroma instrument (Germany). The measurement included both genders without discrimination. The only group excluded from this test was bodybuilders from gyms to avoid odd results that may come from anabolic steroid doping since they were not subject for such test and did not disclosed information if they were on any performance enhancing drugs.

2.5. Measurement of body mass index
The Body Mass Index can be calculated from simple measurements using a person's height and weight. The equation is BMI = kg/m^2 where kg is a person's weight in kilograms and m^2 is their height in meters squared. A BMI of value 25.0 or higher is overweight, while healthy and fitness range is 18.5 to 24.9. Height and weight data were measured and final calculation was recorded.

2.6. Calculation of body fat
To calculate body fat percentage, waist and hip was measured at site, and then the neck measurement was subtracted to determine the circumference value [15].

2.7. Isolation of genomic DNA

Blood samples collected from participants were the source of genomic DNA. A 200 µl of blood was subjected to DNA extraction by (Reliaprep genomic DNA MiniPrep System) produced by Geneaid /Korea according to company technique, concatenation and purity of DNA were measured using nanodrop (Techne /UK). Samples were kept under refrigeration at -20^0 until use. Each sample was given a number indicating the gender, age, and information of the participant.

2.8. Primers used for site amplification

The following primers were used to amplify the site where RS1815739 is located [16]
Forward primer:
5'- CTGGTTGCCTGTGGTAAAGTGGG -3'
Reverse primer:
5' – TGGTCCACAGTAATGCAGGAAGGG-3'

2.9. PCR amplification conditions

PCR amplification conditions were optimized to follow the program of initial denaturation at 94^{0}C for 4 min., and 35 cycle of denaturation at 94^{0}C for 40 sec., annealing at 60^{0}C for 30 sec., and amplification at 72^{0}C for 40 sec. Amplification was concluded for 10 min. at 72^{0}C as final extension. Amplicons resulted from PCR amplification were subjected to electrophoresis in 2% agarose gel with field strength of 7 v/cm for 90 min. to confirm successful amplification before sending them for sequencing by Macrogen, Korea.

2.10. Software used for data analysis

The SAS (Statistical Analysis Software 2012) and DnaSP6 both were used as an independent packages, BLAST RefSeqGene, MSA viewer, ORF finder, Blastx Blastp, and COBALT at https://www.ncbi.nlm.nih.gov , BLAT, and in – genome BLAT at http://asia.ensembl.org/Homo_sapiens/Tools were employed for data analysis. Data obtained were either represented by tables or graphics as given by the website output and programming.

3. Results

3.1. Criteria and specifications of participants

3.1.1. Measurement of body mass index (BMI)

Body mass index (BMI) can be a tool to estimate overweight or obesity, but it does not involve body fatness or health of an individual. Readings of BMI may predict health risk; but a healthcare provider performs further assessments. Such assessments include skin folding, evaluations of diet, physical activity, and family history. According to the BMI criteria, people with a BMI between 25 and 29.9 would be classified as overweight and anyone with a BMI over 30 would be classified as obese [17].
However, athletes may have a high rate of BMI because of high muscle and bone density not increased body fatness.
Table 1 shows body mass index (BMI) and body fat percentage (BFP) of participants distributed according the age, gender, and athletic practice for Physical Education Faculty athletes.

Table 1. Body mass index (BMI) in athletes and control participated in the study from Physical Education Faculty

	Physical Education Faculty students				
Gender	Athletic practice	Age / year	No.	BMI	Body fat percentage
Male	Sprinters (150)	18	30	18±0.4	20±0.75
		20	45	18±0.6	20±0.55
		25	50	22±0.6	20±1.1
		30	25	24±0.3	21±1.1
Female	Sprinters (120)	18	20	20±0.5	23±0.22
		20	25	21±0.4	23±0.54
		25	50	22±0.3	24±0.18
		30	25	23±0.6	27±0.33

	Physical Education Faculty students				
Gender	Athletic practice	Age / year	No.	BMI	Body fat percentage
Male	Power lifters and endurance games (50)	18	10	21±0.5	25±0.12
		20	10	22±0.75	26±0.16
		25	25	22±0.55	26±0.28
		30	5	23±0.8	30±0.13
Female	Power lifter and endurance games (30)	18	5	21±0.45	22±0.33
		20	7	22±0.65	24±0.26
		25	10	22±0.7	26±0.31
		30	8	24±0.21	32±0.11
Control male (50)		18	10	21±0.4	23±0.45
		20	15	23±0.7	23±0.56
		25	10	24±0.8	25±0.78
		30	15	27±0.2*	31±0.21
Control female	Non	18	5	22±0.23	21±0.55
		20	7	22±0.44	24±0.36
		25	7	24±0.21	24±0.88
		30	6	28±0.85*	38±0.14

*= significant increase in BMI with 0.05 degree of freedom.

As given in Table 1, BMI and BFP in all athletes were within normal and healthy ratio. A significant increase was found in both genders control group when they reached age 30 year old, suggesting it came from high calories diet or reduced physical training. In addition, body fat percentage (BFP) was found to increase in 30 years old control, which may increase as the age advances.

3.1.2. Testosterone level, upper arm and thigh muscles circumference

Testosterone represents the key feature hormone in athletic performance for its effect on metabolism, protein synthesis, and fast muscle recovery. This hormone is present in both genders, and it is significantly higher in male than female. However, several report revealed there is an increase in testosterone level in women than the higher ratio occurred naturally [18]. Effect of testosterone is noticeable on parts of the body subjected to stress during athletic performance represented by the upper arm (biceps and triceps) and upper legs (thighs). Such increase in muscle mass is elaborated in Table 2 with testosterone level in both athletic genders.

Table 2. Measurement of testosterone level, upper arm, and thigh muscle circumference in athletes of Physical Education Faculty

Age /year	Athletic practice	Gender	Circumference of the upper arm / cm	Circumference of the upper thigh / cm	Testosterone level (athletes) ng/ml
18	Sprinters	Male	30±0.15	57±1.2	10±0.1
20					
25					

Age /year	Athletic practice	Gender	Circumference of the upper arm / cm	Circumference of the upper thigh / cm	Testosterone level (athletes) ng/ml
30					
18	Power and endurance athletes	Male	36±1.3	61±0.45	10±1.3
20					
25					
30					
18	Control	Male	21±0.12	28±1.3	7±0.75
20					
25					
30					
18	Sprinters	Female	22±1.17	52±0.95	1.62±0.1
20					
25					
30					
18	Power and endurance athletes	Female	31±2.15	55±0.88	1.88±1.89
20					
25					
30					
18	Control	Female	18±0.26	32±1.55	1.1±0.25
20					
25					
30					

Moreover, during recording data regarding muscle circumference, and testosterone level in athletes of Faculty of Physical Education we came across rare odd readings. To confirm that these readings are natural, athletes were subjected to anabolic steroid doping test by professional health practitioner at the college. Test came negative for AAS doping and their results were included in this research and presented in Table 3.

Table 3. Odd readings of upper arm muscle, thigh, and testosterone in athletic students.

Age / year	Athletic practice	Gender	No. of athletes	Circumference of the upper arm/ cm	Circumference of the upper thigh/cm	Testosterone level ng/ml
25	Power and endurance	Male	2	42	66	15
30	Power and endurance	Female	1	33	58	11.5

The reason presenting such odd data is that it may be an indicator for genetic change at *actin3* gene that affect dramatically muscle mass and athletic performance which will revealed in the molecular analysis and genotyping of participants.

3.1.3. Gym athletes participants criteria

Subscribing to gyms and practicing bodybuilding and fitness training is now widely distributes among population regardless age since the problem of obesity is growing to unacceptable ratio resulting in serious health issues. During sample collection, we noticed that there is no limit to age among players, since it started from 12 years old to more than 60 years old. Under age athletes, and players over 50 years old were not included in this study for two reasons: first underage athletes required their parent consent which they did not provide,

and second; senior athletes practice only light workout that does not affect muscle mass or influence body shape. Thus, athletes with age 20 to 50 years old were characterized for BMI and BFP as given in table (4).

Table 4. Body mass index (BMI), body fat percentage (BFP) among bodybuilders distributed according to age groups

Age group / year	No. of athletes	BMI	BFP	No. of control	BMI	BFP
20	25	18±1.4	22±1.2	20	21±0.41	23±0.47
25	30	22±2.1	23±.75	10	23±0.72	23±0.55
30	40	22±0.75	27±1.1	10	24±0.81	25±0.77
35	30	24±0.5	29±1.45	25	27±0.22*	31±0.23
40	25	23±1.2	30±1.36	15	32±0.76*	31±0.67
45	10	24±2.3	30±2.2	10	35±0.86*	35±0.85*
50	10	24±1.7	31±0.95	10	40±0.13*	37±0.25*

***= significant increase in the value compared with control and world standards with degree of freedom 0.05**

1.1.4. Genotyping and allele frequency

Genotyping is performed to determine and analysis of the DNA sequence at specific positions within the genome of an individual. Variations in sequence can be used as markers in the linkage and studies to estimate genes significance to specific traits. In this study distribution and frequency of R, RX, and X was determined among participant and listed in Table 5.

Table 5. Distribution, genotype, and frequency of R and X alleles among participants

						Hardy-Weinberg equilibrium					
Gender	Athletic practice	No. of participants	RR	RX	XX	R relative frequency	RX relative frequency	X relative frequency	577R frequency	577X frequency	X^2
Male	Sprinter	150	75	50	25	66.6667	66.6667	16.6667	0.6667	0.3333	9.375
Female	Sprinter	120	50	50	20	46.875	56.25	16.875	0.625	0.375	1.4815
Male	Power and endurance	50	15	15	20	10.125	24.75	15.125	0.45	0.55	7.7594
Female	Power and endurance	30	6	12	12	4.8	14.4	10.8	0.4	0.6	0.8333
Male	Bodybuilder	170	30	80	60	28.8235	82.3529	58.8235	0.4118	0.5882	0.1388
Male	Control	150	57	62	21	55.3143	65.3714	19.3143	0.6286	0.3714	0.3724
Female	Control	50	12	21	17	5.4516	15.0968	10.4516	0.4194	0.5806	23.3304

3.2. Molecular analysis of *acitn3* among participants

3.2.1. Electrophoresis, sequencing and in – genome sequence location

Specific amplification of desired location in the genome is an essential step in successful data analysis. Using highly specific primers, accurate sequencing and specialized software may reveal much information needed to

be elaborated and may have not been published. Electrophoresis of amplicon resulted from PCR is given in Figure 1.

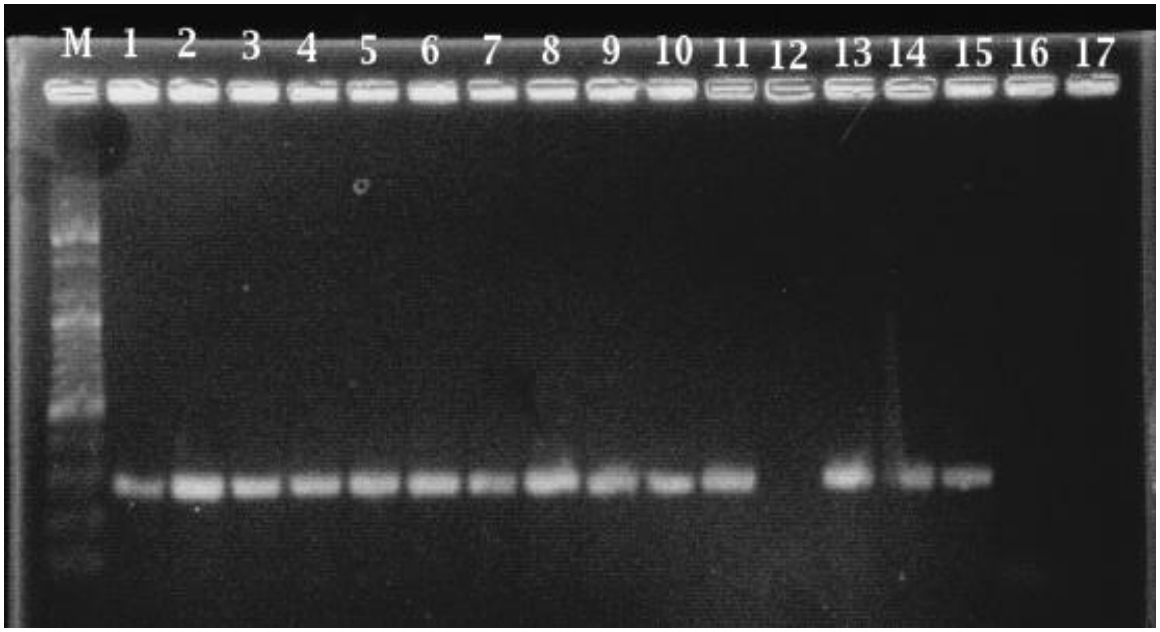


Figure 1. Gel electrophoresis of *actin3* amplicons selected from PCR amplification of genomic DNA extracted from participants. M is DNA marker (100 – 1500 bp), lanes 1 and 2 from male sprinters; lane 3 and 4 from power and endurance athletic male, lanes 5 and 6 from sprinter female, lanes 7 and 8 from females athletes of power and endurance, lanes 9 and 10 from bodybuilders males, lanes 11, 13, 14, and 15 represent control of male and female respectively.

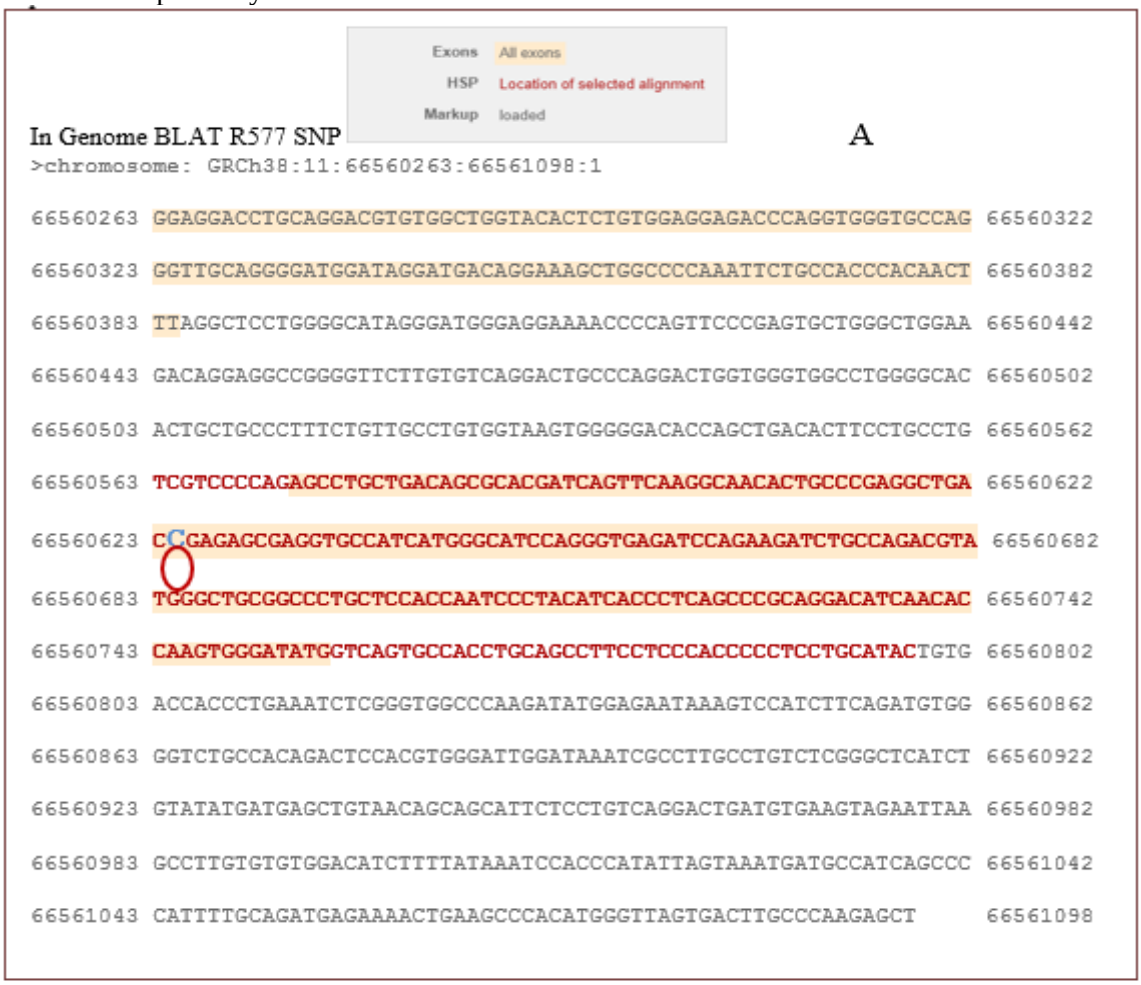


Figure 2. In – genome sequence BLAT using BLAT tool available at http://www.ensembl.org/Multi/Tools/Blast. Figure shows location of R577 SNP given by large letter with blue color, while B shows 577X polymorphism given by the same letter. The location of this polymorphism is at 66560624 on Chr 11.

Resulting in – genome sequence data BLAT is shown in Figure 2 which represent the location of sequences from *actin3* amplification on the chromosome.

Figure 2 shows the presence of SNP at position 66560624 (C or T) from which genotypes can be identified. Presence of such SNP may determine whether *actin3* can be fully transcribed or subjected to premature stop codon [19]. Moreover, many literatures [1] [3] [20] referred that R577X lies on exon 16. However, our finding resulting from multiple sequence alignment with consensus sequence located this SNP to be at exon 15 as shows in Figure 3.

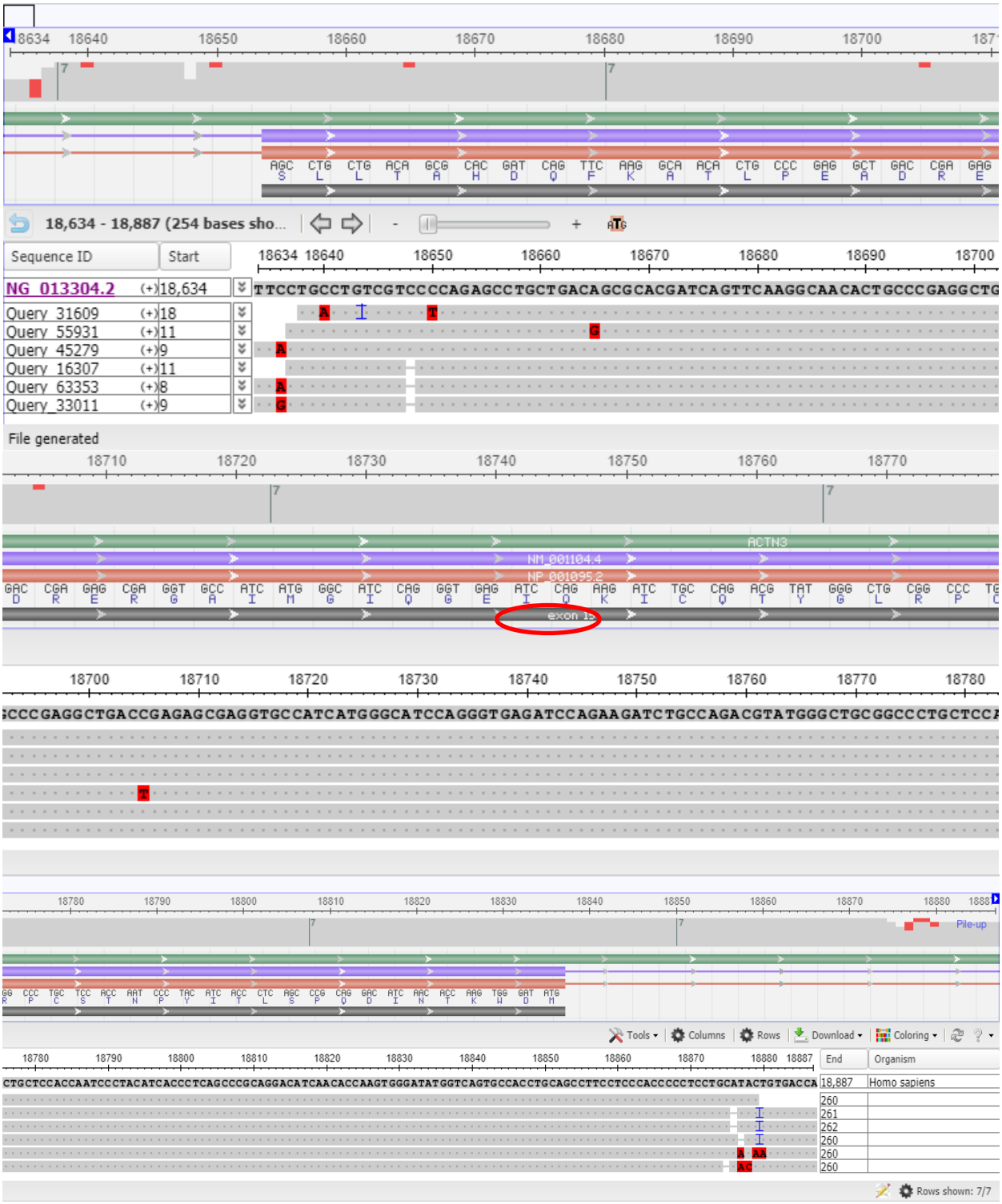


Figure 3. Multiple sequence alignment of sample of participants for *actin3* gene. The figure shows that location of R577X lies within exon 15. Red marked nucleotides represent differences among sequenced amplicons.

3.2.2. Transcription site and Open Reading Frames (ORFs)

Open Reading Frames (ORFs) are critical sites from which DNA transcription stars. Presence of ATG start codon determines the +1 codon for RNA polymerase to start DNA transcription. Among participants, presence of different genotypes may alter such process of expression; either the gene is fully transcribed or subjected to pre – mature termination. Analysis of ORFs for participants with different genotypes showed three types of them, one where is fully transcribed, second where the gene is transcribed but with additional ORF, and third where the ORF is terminated at 577X. Elaborated detailed analysis is shown in Figure 4.

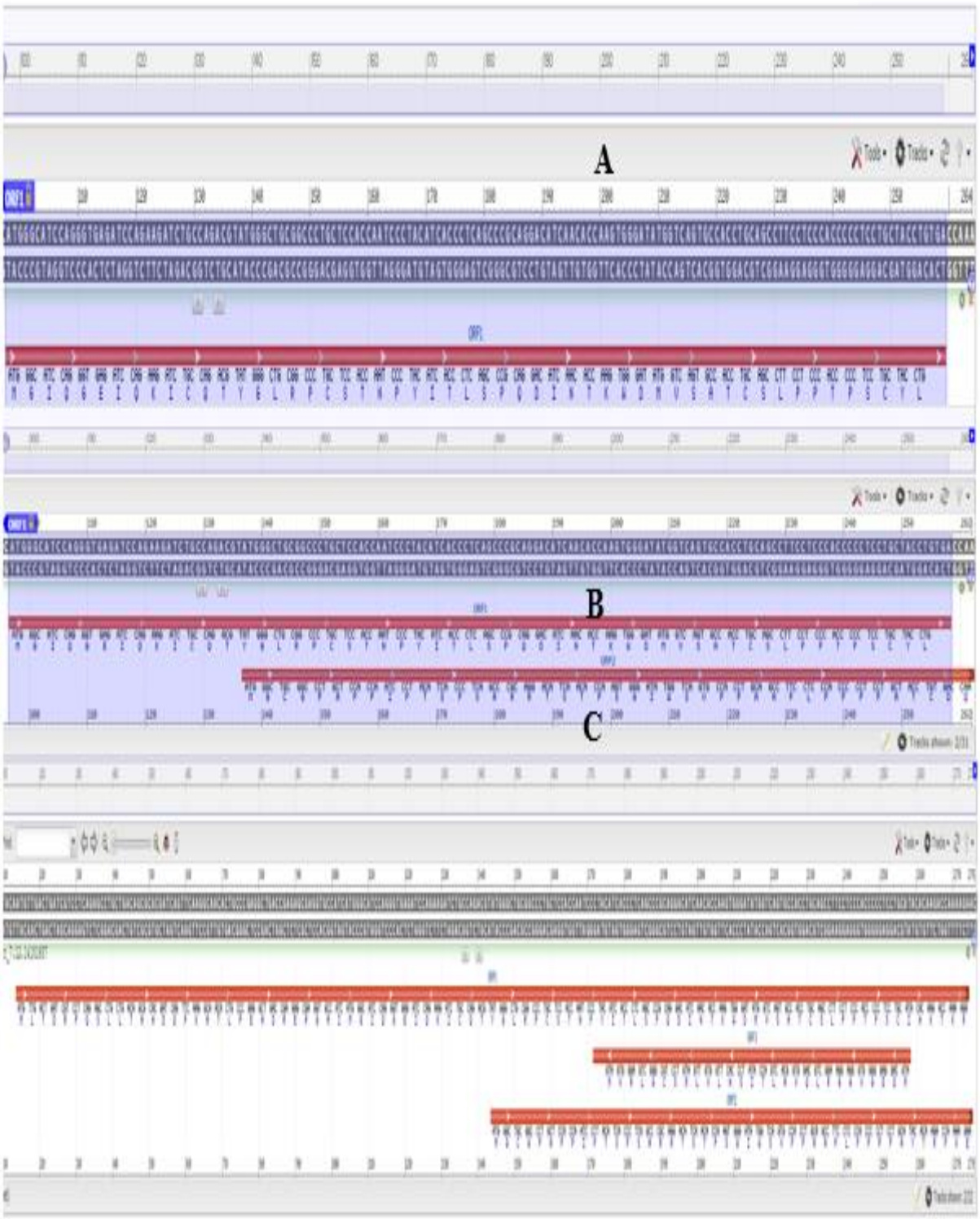


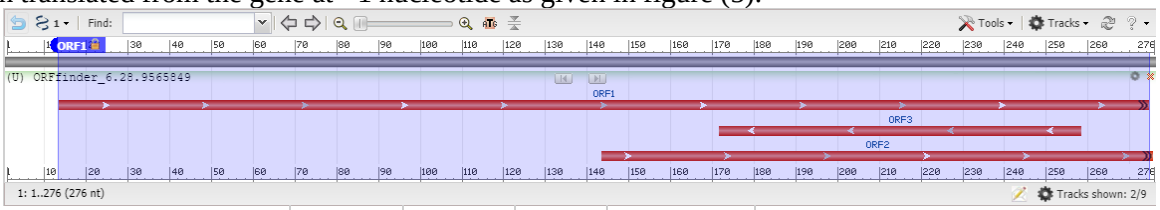
Figure 4. Open reading frames of actin3 gene. A rpresents ORF of 577X homozygous genotype, B represents ORF of R577X heterozygous genotype, and C represents ORF of 577X homozygous genotype.

Figures 3 and 4 can elucidate criteria that may be found within *actin3* gene first: there are multiple polymorphic sites at different positions of the gene. In our case they were located at 18634, 18640, 18650, 18655, 18704, 18878, and 18880. Presence of such polymorphism did not affect gene function or altered its expression, but can provide an insight for gene evolution, difference in impact on physical and performance of an individual toward intensive training, hard work and labor unlike figure (4) that can explain gene expression in either case since it involves ORFs that control protein synthesis from the gene which is incorporated in muscle fibers.

3.2.3. Analysis of odd finding of the gene composition

During our study, among all participants involved, 3 of them (2 males and 1 female) were found to exhibit higher levels of muscle fibers (bigger muscles), testosterone, and exceled physical and athletic performance. All three of them were found to be 577X homozygous type of *acint3* gene with different polymorphic DNA patterns.

Expression from ORF of the 3 excelled athletes took a different path. Such difference extended to different protein translated from the gene at +1 nucleotide as given in figure (5).



Label	Strand	Frame	Start	Stop	Length (nt | aa)
ORF1	**+**	**2**	**14**	**>274**	**261 | 86**
ORF2	+	3	144	>275	132 | 43
ORF3	-	1	258	172	87 | 28

Figure 5. ORFs and positions from which gene expression begins in tree excelled athletes with different characteristics. All three of them were found to XX homozygous genotype.

3.2.4. Protein BLAST

Different expression patterns may not mean different protein, but in this case the different in protein was significant when compared to their companions with the same genotype. Protein BLAST is given in figure (6)

Query 4 DRPQSLLTAHDQFKATLPEADRERGAIMGIQGEIQKICQTYGLRPCSTNPYITLSPQDIN 63

Sbjct 144 **EET**..... 203

Query 64 TKWDMV 69

Sbjct 204 209

Figure 6. Protein BLAST from *actin3* among athletes with different characteristic. It shows a significant difference at three positions at the beginning with 5 extended amino acids at the end. More detailed analysis is given in figure (7) which elucidates nucleotide different in investigated genotype.

Query 18 TGACTGATCGTCCTCAGAGCCTGCTGACAGCGCACGATCAGTTCAAGGCAACACTGCCCG 77

Sbjct 18638 ..**C**...**-**.....**C**..... 18696

Query 78 AGGCTGACCGAGAGCGAGGTGCCATCATGGGCATCCAGGGTGAGATCCAGAAGATCTGCC 137

Sbjct 18697 18756

Query 138 AGACGTATGGGCTGCGGCCCTGCTCCACCAATCCCTACATCACCCTCAGCCCGCAGGACA 197

Sbjct 18757 18816

Query 198 TCAACACCAAGTGGGATATGGTCAGTGCCACCTGCAGCCTTCCTCCCACCCCCTCCTGCA 257

Sbjct 18817 18876

Query 258 TAC 260

Sbjct 18877 ... 18879

Figure 7. Nucleotide difference in the genotype under investigation. The figure shows difference at position 18640, 18644, and position 18650.

3.2.5. Significance of the genetic change

In many genetic studies, DNA polymorphism is the key to genotyping. However, not all genetic and DNA polymorphism may impact or alter gene function. This requires several parameters need to be investigated to give such assumption.

3.2.6. DNA polymorphism

DNA sequence from participants with different characteristics was compared with their companions to illustrate DNA polymorphism. Data obtained showed that Number of polymorphic (segregating) sites, S: 5 with total number of mutations, Eta: 5, gene diversity, Hd: 1.000, Variance of Haplotype diversity: 0.01600, Standard Deviation of Haplotype diversity: 0.12, the average number of nucleotide differences per site between two sequences (Nucleotide diversity) Pi: 0.44000

3.2.7. Synonymous and Nonsynonymous Substitutions

The total number of synonymous and nonsynonymous sites is calculated by [21]. Silent sites refer to both synonymous sites and the noncoding positions. Synonymous sites are codons where nucleotide changes result in synonymous substitutions. Ks (the number of synonymous substitutions per synonymous site) for any pair of sequences. Results obtained are sown in table (6).

Table 6. Synonymous and non-synonymous substitutions among DNA sequences obtained from *actin3* for athletes with excelled characteristics compared to their colleagues.

Seq 1 athletes with XX genotype	Seq 2 athletes with distinct characterizes	SilentDif	SilentPos	Ks
'ref|NG_01	'lcl|Query	2.00	5.00	0.5716
'ref|NG_01	'lcl|Query	1.00	5.00	0.2326
'ref|NG_01	'lcl|Query	1.00	5.00	0.2326
'ref|NG_01	'lcl|Query	2.00	5.00	0.5716
'lcl|Query	'lcl|Query	3.00	5.00	1.2071
'lcl|Query	'lcl|Query	3.00	5.00	1.2071
'lcl|Query	'lcl|Query	4.00	5.00	n.a.
'lcl|Query	'lcl|Query	2.00	5.00	0.5716
'lcl|Query	'lcl|Query	3.00	5.00	1.2071
'lcl|Query	'lcl|Query	1.00	5.00	0.2326

3.2.8. Analysis of codon bias

Codon usage bias indicates the differences in the frequency of occurrence of synonymous codons in exons. The abundance related to the number of codons allows many amino acids to be incorporated by more than one codon. The genetic codes are often biased towards using one of the several codons that encode the same amino acid over the others that is, a greater frequency of one will be found than expected by chance [22]. Determination of codon bias is illustrated in Table 7.

Table 7. Codon bias among athletes obtained from analyzing DNA sequence of *actin3* gene between athletes with different and exceled characteristics and their colleagues

Ref sequence	ENC	CBI	$SChi^2$
'ref|NG_013304.	1.000	1.000	n/a
'lcl|Query_5842	1.000	1.000	n/a
'lcl|Query_3258	1.000	1.000	n/a

'lcl|Query_1491	1.000	5.000	n/a
'lcl|Query_1555	1.000	5.000	n/a

ENC, Effective Number of Codons, CBI, Codon Bias Index, SChi2, Scaled Chi Square

3.2.9. Linkage disequilibrium

In a population genetics, linkage disequilibrium (LD) is the non-randomized association of alleles at different loci. Loci are said to be in disequilibrium linkage when the frequency of association of their different alleles is higher or lower than what would be expected if the loci were independent and associated randomly [23]. Estimation of LD is given in table (8) for athletes participated in this study compared to those with different criteria of athletic performance.

Table 8. Linkage disequilibrium in nucleotide sequence as nonrandom association between nucleotide variants at different polymorphic sites. investigated for excelled athletes compared to their colleagues.

Site1	Site2	Dist	D	D'	R
1	2	1	-0.080	-1.000	-0.408
1	3	2	-0.040	-1.000	-0.250
1	4	3	-0.040	-1.000	-0.250
1	5	4	0.160	1.000	1.000
2	3	1	0.120	1.000	0.612
2	4	2	-0.080	-1.000	-0.408
2	5	3	-0.080	-1.000	-0.408
3	4	1	-0.040	-1.000	-0.250
3	5	2	-0.040	-1.000	-0.250
4	5	1	-0.040	-1.000	-0.250

D= the linkage disequilibrium determinant, D’ is the relative value of disequilibrium, Dist. Is genotypic distribution at equilibrium, and R is recombination fraction between the loci.

4. Discussion

The α-*actin3* is a part of protein family spectrin, which includes dystrophin [24]. In human skeletal muscle, the two sarcomeric α-*actin3* isoforms, α-*actin2* and α-*actin3* construct major components of the contractile mesh at the Z-line [25]. Both isoforms are conserved and are considered products of gene duplication [26]. They have common domain topography, made of a stringently conserved N-terminal actin-binding domain, as a central rod domain consisting of four spectrin-like repeats, and a C-terminal EF hand region [27].

Among population, genes may play a crucial role in performance, but lacking physical tendency, or highly responsive nerve, and strong cardiac function may fail them as athletes. Thus, individuals with optimized physiology accompanied with desired genes are the perfect candidates for athletic performance.

Physical fitness in participants in this study was measured. BMI in all athletes was found within acceptable ratio, regardless the elevation of the record for some athletes, especially those practicing power and endurance sports. This may be attributed to higher bone and muscle density as a natural response to the stress resulting from pushing high weights and healthy diet. Such results were confirmed with BFP that falls within fitness ratio for all athletes compared with control who showed elevation in body fat percentage as the age progress as a result of unhealthy diet and lack of physical training. Such results were found to be less in bodybuilders due intense training, committing to low fat high protein diet and practicing fitness training to cope with bodybuilding training.

Studying R577X distribution among athletes may not be a precise indicator for such distribution among population. This may regarded to the reason that athletes pursue the sport that may excel with, either sprinters or endurance depending on their test results. However, the case bodybuilding may give the same outcome, since the remaining trainees were found to be of XX homozygous genotype followed by RX, with less RR genotype. This is due the change in physical shape among athletes that should be noticed after long period of training.

Lack of such change pushed athletes to drop training and leave gyms. Thus studying the distribution of rs1815739 among population will require random sampling from athletic and non-athletic individual with number exceeds 2000. No differences were found between controls and participants in the ACTN3 R577X allele/genotype distribution. The genotype XX is a result of complete protein deficiency and the Actn3-/- MSTN and ACTN3. Polymorphism in athletes shows a shifting in the properties of fast fibers towards a more oxidative phenotype [28]. Thus, it is possible that the X-allele could confer some resistance against metabolism-related diseases and contribute partially, to extend life expectancy in some individuals [29].

Testosterone is normally circulating the body of both genders. Due the regulation of endogenous testosterone production, including the acute influence of competition and exercise, testosterone concentrations may grow considerably within and among individuals [30]. In athletes, testosterone level was found to be elevated than normal values compared to control in both genders with significant value. This is attributed to stress, and muscle damage during athletic practice which inflected a physiological response toward such stress. In other report [31] suggested that deficiency in *actin3* may trigger an elevated testosterone in both genders especially with genotype XX.

Athletic performance involves complex criteria affected by heredity, exercising, diet, and socio-demographic factors. Normally, there has been an idea that acknowledges the influence of environmental factors to reach the level of elite athlete, although it seems that some athletes are naturally talented for certain sport types. In this regard, recently, evidence suggesting that genetics plays a vital role in athletic performance has increased exponentially and the influences of genetics and environment on the ability to excel in sport are well recognized [32]. A more analytical view will show among high performance athletes, only few will excel others due multiple physiological and genetic factors. In our case, among 520 participants, two males and one female were found with such criteria. Genetic analysis of these athletes showed a significant change in their *actin3* sequence represented by +1 codon which gave different amino acids when translated at the beginning and the end. Such difference was also accompanied with alteration at the same exon represented by transition, Indel, transversion 18640, 18644, and 18651 respectively. Changing proteins translated (figure 7) altered the gene function dramatically rendering the gene inactive in muscle building giving the chance to dramatic events to be triggered represented by unbind muscle growth, and inducing high testosterone level within body of athletes. This may induce athletic performance in these athletes toward excelling others with high scores in their sport. This genetic change was found to be significant when DNA polymorphism, Synonymous and Nonsynonymous Substitutions, codon bias, and linkage disequilibrium was tested.

5. Conclusions

Alpha – *actin3* 3 gene involves in one way or another in athletic performance. As reported, it is inactive in 16 – 18 % of the population without any phenotypic disorder, since it is compensated by other genes direct muscle formation like actin2. The gene comprises three genotypes RR homozygous, RX heterozygous, and XX homozygoys depending on specific SNP which either make the gene function completely (RR, and RX genotypes) or render it inactive due to mature stop codon (XX genotype). Among participants involved in this study, no significant difference was found among genotypes with statistical calculation, however, XX genotype was found less than the other. The important findings during this study is that first: the location of R577X (rs1815739) was found on exon 15 not exon 16 as cited by previous literature. This confirmed with multiple sequence alignment with consensus sequence. Second: odd SNPs with high significance were identified. These have a dramatic impact on muscle formation especially in exceled athletes since they developed higher muscle density, higher and healthy BMI with low BFP, and high testosterone level as a physiological response against intensive training. Those athletes were with XX genotype.

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