Determination the genetic diversity of the Actinin-3 gene as a function of selection for Egyptian players at senior levels in the sport of weightlifting

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Abstract: The genetic diversity of Actinin-3 gene (responsible for the formation of proteins association muscle fibers) of the higher levels players in the sport of weightlifting was identified as a function of selection, and to study the relationship between the alternative allele of Actinin-3 gene (R577R) and the level of achievement of the higher levels players in the sport of weightlifting. DNA was analysis by polymerase chain reaction (PCR) to make amplification of Exon-16 in Actinin-3 gene using two specific DNA primers followed by partial digestion using restriction enzyme (Dde1) specialized to detect alleles of Actinin-3 gene (R577R and R577X). The results of PCR amplification for the target part of Actinin-3 gene were similar in size of amplified fragments which means that, whatever the genotype of this region of the gene, nucleotide changes were not due to loss or gain genetic material in the gene but they were due to the changing nature of the linear sequence (nucleotide substitution) of nucleotides in this region. The partial digestion of the amplified fragments of exon-16 (290 bp) of the homozygous genetic pattern (RR) resulted in two fragments (85 and 205 nucleotides) due to the presence of one cut position. Meanwhile, three fragments were resulted from the homozygous genetic pattern (XX) with size of 86, 97 and 108 nucleotides due to presence of two cut positions. While, the digestion analysis of the heterozygous genetic pattern (RX) resulted in five fragments, three of them were 86, 97 and 108 nucleotides, specialized to style (X), in addition to two fragments (85 and 205 nucleotides) specialized to style (R). The results show that the heterozygous genotype (RX) has the largest percentage rate (50%) in the players sample and the homozygous genotype (RR) ratio reached its presence in the sample (30%), while the genotype (XX) ratio reached its presence in the sample (20%). The distribution of the genotypes (RR-RX-XX) of Actinin-3 gene in the sample was in ratio (1:2:1) which means the heritability of Actinin-3 gene follows the simple mendilian's traits inheritance, which inherited between individuals without abnormalities. The results of statistical analysis show presence of significance moderate forward correlation between the genotypes of Actinin-3 gene (RR-RX-XX) and the achievement level of the higher levels players in the sport of weightlifting. Also, presence of significance moderate forward correlation between the two genotypes of allele-R (RR and RX) and the level of weightlifting, with no significance differences between homozygous genotype (RR) and heterozygous genotype (RX). Moreover, the results show the presence of differences between genotypes (RR) and (XX) which reflect the dominance of allele (R) on allele (X), where the owners of these two genotypes have muscles stronger and faster than the owners of genotype (XX), which reflect the correlation between (R577R) allele and the higher levels of achievements of the players. So, it can rely on the genotypes (RR) and (RX) in the selection of members of sports depends on the strength and speed significantly.

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1.Introduction:

During the past years, the field of sports physiology has been shown a lot of achievements as a results of using the modern technologies, such as the use of radioactive materials to study the cellular metabolic processes at rest and under conditions of different exercises in addition to use of magnetic resonance to identify the muscle metabolism in various sports activities. At the top of these modern technologies techniques of molecular biology and genetic engineering that consider unprecedented revolution in directing human performance, and that will lead to mutation in sport sciences because it will enable us in the future to reach the individual to ideal genetic specifications to cope with the rapid progress on a global level. Many researches conducted, by researchers of the Australian institute of sport in the department of genes clinical beside to other

researchers in the world, to try to identify genes help predict nature ability kinetic using the analysis of DNA for the higher levels players to insight the genetic differences. These researchers have discovered that rowing players have genetic code help them on the health of the circulatory system. MacArthur and North (2004) found that genes controlled by (30%) of the responses of the heart muscle for athletic training. Also, Homann and Seedel (2003) reported that the gene responsible for about (50%) of the differences between human beings in maximum oxygen consumption. And both Neil Spiroa and Henning Walk (2006) agreed that gene play a role in muscle fiber type representing (45%) and gene affect the increased vulnerability anaerobic exercises. Konalakis *et al.* (2002) made study on 40 of the twins and reported that the genetics has a role of (91 %) of the maximum pulmonary ventilation, as well as for the inspiratory flow rate for twin's cases participate in the search. One of the most important scientific achievements in the field of genetic link to performance sports completion of the so-called "Human genome map performance and genetic variations of health-related fitness" which began monitoring in the year 2000, as well as the map of the human genome for obesity which start monitoring in 1999.

Muscle fibers consist of long tubes called muscle fiber, which are comprised of filaments that are of two kinds: thin type is actin protein and thick type is myosin protein, they are parallel together. The actin filaments are installed by association of actin proteins known as Actinin (Actinin-2 and Actinin-3). Alpha Actinin (α-Actinin) represents the main group of association proteins for the F-actinin that present in both muscle and non-muscle cells. There are four genes coded for α-actinin proteins, two of them are non-muscle (α-actinin-1 and 4) and the other two are muscle (α-actinin-2 and 3) (Biggs *et al.*, 1992; Yorocker and Negila, 1992; Sue *et al.*, 2004). The actinin-2 protein exists in all skeletal muscles, while the actinin-3 protein found only in fast contraction skeletal muscle which is also known as α-actinin skeletal muscle type-3 or interface protein link to F-actinin (North *et al.*, 1999; Mikerther and North, 2004; Yang *et al.*, 2003). The α-actinin-3 that link actin has different roles according to cell type and its gene expression will be only in skeletal muscle. It is located at Z-disk and corresponding dense objects, which helps to stabilize systolic fibroblasts actin filaments (Biggs *et al.*, 1992; Achan *et al.*, 1998). Actinin-3 gene located in long arm of chromosome 11 at (11q13.1) band and consists of 22 exons with total size 16407 pairs of nucleotides, from the point of 66314391 to 66330797 along the chromosome. In human there are several patterns of Actinine-3 gene result from changing of nitrogen bases in many places of the gene, which are often found in at least 132 positions due to genetical and environmental mutations in advanced times of human life on earth. The most famous mutation is (R577X) that means turning the arginine code to stop code causing instability of messenger RNA (m RNA) and amputations of translated protein. This mutation caused a significant shortfall in the amount of actinin-3 in the holders (20-25% of people around the world). Overall, the Africans are showing the lowest level of the presence of this mutation, while the Asians show the highest percentage of its presence. Sports research has linked between Actinin-3 and speed defibrillation. As the selection process of rookies are traditional methods where they rely on some anthropometric measurements, which may give unhonest indications, especially in the early stages.

So this study aims to identify the gene help predict natural sports capacity, and determine the genetic diversity of the Actinin-3 gene for higher levels players in the weightlifting sports as a function of selection. Also, study the relationship between the alternative allele (R577R) of Actinin-3 gene and achievement level of the higher levels players in the sport of weightlifting. Moreover, to make particular genetic frame for capabilities of various sports to guide the individual to allocate in sports fits his abilities and for selection the rookies according to their genetic aptitudes. As well as, the possibility of applying it to selection of individuals to take over private businesses require certain physical capabilities, such as the selection of individuals to join the colleges and institutes of military, security and sporty, and then selecting personals for army units require high physical capabilities, as selection members of the special forces units. It should by noted that this study conducted on this segment of the Egyptian players heroes for the first time by using PCR technique followed by partial digestion using one restriction enzyme specialized for detecting alleles of Actinin-3 gene as a function of selection for future players of sports.

2.Material and Methods:

The study involvement ten Egyptian players who hold international tournaments in the sport of weightlifting (Table 1). Tournaments have been identified and centers achieved in each tournament as well as the weight of each players (Table 2).

Genetic analysis was conducted using 4ml blood sample and the genetic material (DNA) was isolated according to the method of "Helms, 1990 " with some modifications. The amplifications of Exon-16 of Actinin-3 gene were achieved by polymerase chain reaction (PCR) using two specific DNA primers:

Forward (5'-CTGTTGCCTGTGGTAAGTGGG-3') and reverse (5'-

TGGTCACAGTATGCAGGAGGG-3'). The PCR mixture (25 µl) consists of 100 ng extracted DNA, 1.5 mM $MgCl_2$, 200 µM of each dNTPs, 1x reaction buffer and one unit of Taq DNA polymerase enzyme in addition to bidisteled water. The PCR conditions were as follows: one time of preheating at 94°C for 5 minutes then 35 cycles of: denaturation step at 94°C for one minute, annealing step at 58°C for 30sec. and extension step at 72°C for 40 sec. followed by heating at 72°C for 5 min to enable all the incomplete pieces to complete their length. PCR products were tested on agarose gel (1.5%) figure 1. Partial digestion was conducted for PCR products using (Ddel) enzyme which incubated with 20 µl of the PCR products for ten minutes at 38°C. The partial digestion products were tested on an agarose gel (2%) figure 2. The data obtained was statistically analyzed by arithmetic mean, the mediator, the percentage, the correlation coefficient and Chi-square (χ^2) test.

3. Results and discussion:

The exon-16 of Actinin-3 gene was amplified for ten Egyptian players who hold international tournaments in weightlifting sport by PCR using two specific primers. The PCR products were separated on the gel (Fig.1) that showed the amplified fragments (bands) of the target part (exon-16) of Actinin-3 gene which were similar in size (290bp) that means whatever the genotype of this region of the gene, changes nucleotide were not due to loss or gain genetic material in the gene, but were due to the changing nature of the linear sequence of nucleotides in this region (nucleotide substitution). To determine the genotype of the genetic changes in this expression region (exon-16) from Actinin-3 gene, the amplified fragments resulting after maximizing by PCR were partial digested using Dde1 restriction enzyme. The products of digested reaction were separated on the gel (Fig.2) and the molecular weight of these products are presented in table (1). The results show that the (R557R) allele was cut into two fragments with size of 205 and 85 nucleotides due to presence of one cut position of Dde1 enzyme. Also, the (R557X) allele was cut into three fragments with size 108, 97 and 86 nucleotides due to presence of two cut positions of Dde1 enzyme. Thus, the partial digestion analysis of homozygous genotype pattern (RR) showed two fragments (205 and 85bp) and for homozygous genotypes pattern (XX) showed three fragments (108,97 and 86 bp). While, the results of partial digestion of heterozygous genotype pattern (RX) showed five fragments, three of them in size of 108, 97 and 86 bp in addition to two fragments in size of 205 and 85bp.

Because the sensitivity of the gel is weaken with the smaller fragment, so the fragments of size 97, 86 and 85 bp will merge in one fragment, thus the resulted fragments of (RR) and (XX) patterns were two fragments, while it were of (RX) pattern three fragments (Table 1). The heterozygous genotype (RX) occupied the largest percentage rate of (50 %), and the homozygous genotype (RR) reached ration of (30%) in the sample, while the genotype (XX) presences in ratio of (20%). The results of statistical analysis showed presence of significant positive medium correlation (0.42 at significance > 0.01) between the genetic diversity (RR-RX-XX) and the rate of greatness (the directory efficiency weightlifter, higher elevation of player to his weight, Table 2), these results are consistent with the results of Nan Yang *et al.* (2003) which reported the existence of a relationship between the different types of Actinin-3 gene and the elements of speed / power muscle.

Also, the results showed the presence of significant different towards allele (R557R) by ratio reached (80%) and presence of significant positive medium correlation (0.55 at significance > 0.01) between both genotypes (RR and RX) of allele (R577R) and the rate of dignity. This result are in agreement with the study of Moran *et al.* (2007); and also are in agreement with the study of BaBa demetrio *et al.* (2008); and Droswescaa *et al.*(2008), where they decided presence a strong correlation between the presence genetic diversity of (RR-RX) and excel in activities of the force special by speed.

The results of Chi square test (χ^2) for the distribution of genotypes (RR-RX – XX) showed that the value of calculated χ^2 (2.1) was less than the value of tabular χ^2 (5.99) which shows that the percentage of genotypes distribution (RR –RX –XX) of Actinin-3 gene in the sample is (1:2:1) that indicates the Actinin-3 gene followed in its inheritance the pattern of simple Mendelian`s characters that transmitted between individuals without abnormalities. Despite this, there are some exceptions, where two individuals of the research sample who have the genotype (XX) achieve advanced positions in many international tournaments. This was due to the fact that many of the varied genes control defibrillation and its efficiency, thus inheriting this complex structure will be a complexity analysis and interpretation of the overlapping all, where must by study other types of genes associated the muscular constriction and also study large and more diverse samples. May also this due to psychological reasons

(psychological fluency – motivation) and environmental reasons (such as growing up in a favorable athletes climate). This is consistent with the study of Hopkins (2001).

Conclusions:

The results showed the presence of positive correction between different genotype of the Actinin-3

gene (RR-RX-XX) and the level of athletic performance for players at senior levels in the weightlifting sport. As well as the existence of significant positive correlation between allele (R577R) of Actinin-3 gene and the level of players achievement with no significant differences between homozygous genotype (RR) and heterozygous genotype (RX). While the results showed presence of differences between the genotype (RR) and the genotype (XX) which reflect the dominance of allele (R) on allele (X), thus the genotypes (RR) and (RX) are identical, where the owners of both types (RR) and (RX) have muscles stronger and faster than the owners of genotype (XX). So, it can rely on types (RR) and (RX) in the selection of members of the sports depend on force and speed significantly. Finally, it can rely on the genetic analysis of Actinin-3 gene to select members of sports rely on strength significantly.

Table (1): Genotypes characterization of Actinin-3 gene for members of the research sample.

Number	Players name	Molecular weight of DNA fragments resulting from partial digestion by restriction enzyme (Dde1)	Genotypes
1	Imad Abdel Hamid	205 + 108 + 97 + 85	RX
2	Mahmod Ahmed Mahmod	205 + 85	RR
3	Raaft Galal Ali	205 + 108 + 97 + 85	RX
4	Nasser Galal Ali	205 + 108 + 97 + 85	RX
5	Gamal Mohamed Hassanein	108 + 97 + 86	XX
6	Abd el Nabi Zaki Desoqy	205 + 85	RR
7	Ahmed Zeen Mahmod	108 + 97 + 86	XX
8	Nasser Maher Ghazal	205 + 85	RR
9	Ahmed Samir Ahmed	205 + 108 + 97 + 85	RX
10	Hany Samir Riad	205 + 108 + 97 + 85	RX

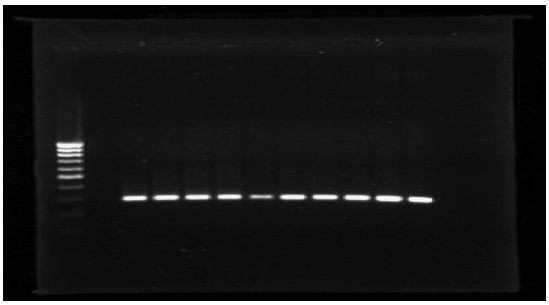

Figure (1): PCR fragments (bands) of Actinin-3 gene (Exon-16). From left: molecular ladder, then players sample from 1 to 10 sequentially as table (1).

Table (2): The genotypes of Actinin-3 gene in comparison of players achievements

Number	Player name	Lifter weight Kgr.	The highest number achieved Kgr.			Ratio of weight lifter to lift	Genotype
			Kidnap-ping	Klein	Total		
1	Imad Abdel Hamid	67	127	160	287	4.283	RX
2	Mahmod Ahmed Mahmod	74	130	170	300	4.054	RR
3	Raaft Galal Ali	76	152	182	334	4.39	RX
4	Nasser Galal Ali	81	145	185	330	4.074	RX
5	Gamal Mohamed Hassanein	76	130	170	300	3.947	XX
6	Abd el Nabi Zaki Desoqy	82	147	185	332	4.048	RR
7	Ahmed Zeen Mahmod	58	100	125	225	3.879	XX
8	Nasser Maher Ghazal	83	150	185	335	4.036	RR
9	Ahmed Samir Ahmed	77	152.5	195	347.5	4.51	RX
10	Hany Samir Riad	93	126	157	290	3.118	RX

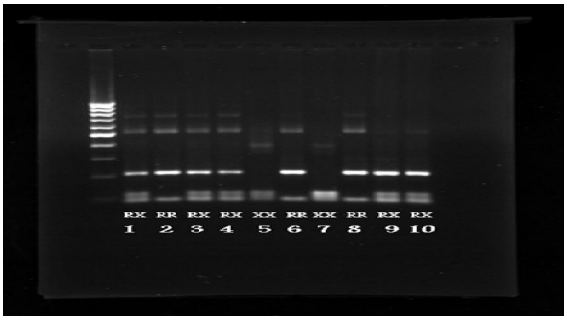

Figure (2): The genetic patterns of Actinin-3 gene determined by partial digestion of Exon-16 using restriction enzyme Dde1. From left: molecular ladder ,then players sample from 1 to 10 sequentially as table (1).

References:

1. **Beggs AH.; T.J. Byers; J.H.M. Knoll; F.M. Boyce; G.A.P. Bruns and L.M. Kunkel (1992).** Cloning and characterization of two human skeletal muscle α-actinin genes located on chromosomes 1 and 11. J. Biol. Chem., 267, 9281–9288.
2. **Chan Y.; Tong HQ.; Beggs A.H. and Kunkel L.M. (1998).** "Human skeletal muscle-specific alpha-actinin-2 and -3 isoforms form homodimers and heterodimers in vitro and in vivo. Biochem. Biophys. Res. Commun. 248
3. (1): 134–9.
4. **Druzhevskaya AM.; Ahmetov II; Astratenkova IV and Rogozkim VA. (2008).** Association of the ACTIN3 (R577X) Polymorphism With Power athletes Status in Russians, sports Genetics Laboratory, Stpetersburg Research Institute of Physical Culture, to Dynamo Ave., 197110, St Petersburg, Russia Eurj Appl Physiol, May 10, 2008.
5. **Helms, C. (1990).** Manual Isolation of Human DNA from Lymphoblasts or whole blood: RFLPs Project (1989) RFLPs, England.
6. **Hohmann, A. and Seidel, I. (2003).** Scientific aspects of talent development International Journal of Physical Education. 40(1):9-20.
7. **Hopkins, W.G. J. (2001).** Genes and training for athletic performance, Sport science, 5(1).
8. **MacArthur DG and North KN (2004).** A gene for speed? The evolution and function of alpha-actinin-3. Bioessays26 (7): 786–95.
9. **Moran C.N.; Yang N.; Bailey M.E.; Tsiokanos A.; Jamurtas A.; MacArthur DG.; North K.; Pitsiladis Y.P. and Wilson R.H. (2007).** Association analysis of the ACTIN3 R577X polymorphism and complex quantitative body composition and performance phenotypes in adolescent Greeks". Eur. J. Hum. Genet. 15 (1): 88–93.
10. **Neil Spurway and Henning Wack Earth (2006).** Genetics and Molecular Biology of Muscle Adaptation, 1st edition. Elsevier, Churchill Livingstone, The British Association of Sport & Exercise Science, London.
11. **North KN.; Yang N. and Wattanasirichaigoon D. (1999).** A common nonsen mutation results in alpha-actinin-3 deficiency in the general population.". Nat. Genet. 21 (4): 353–4.
12. **Papadimitriou I.D.; Papadopoulos C.; Kouvatsi A. and Triantaphyllidis C. (2008).** The ACTIN3 gene in elite Greek track and field athletes. Int J Sports Med 29 (4): 352–5.
13. **Su A.I.; Wiltshire T. and Batalov S. (2004).** "A gene atlas of the mouse and human protein-encoding transcriptomes". Proc. Natl. Acad. Sci. U.S.A. 101 (16): 6062–7.
14. **Yang N.; MacArthur D.G.; Gulbin JP.; Hahn A.G.; Beggs A.H.; Easteal S. and North K. (2003).** ACTN3 genotype is associated with human elite athletic performance. Am. J. Hum. Genet. 73 (3): 627–31.
15. **Yürüker B. and Niggli V. (1992).** Alpha-actinin and vinculin in human neutrophils: reorganization during adhesion and relation to the actin network. J. Cell. Sci.. 101 (Pt 2): 403–14

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