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Evolutionary analysis of actinins(ACTN) gene family for positive selective sites by finding the dn/ds (ω) value

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Authors' Contribution | **Din, I. U, L. Sun & L. Hu** have equal contribution in this research.

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ABSTRACT

Identifying positively selected amino acid sites is an important approach for making a conclusion about the function of proteins; an amino acid site that is undergoing positive selection is likely to play a key role in the function of the protein. Here, we used codon-based substitution models and maximum-likelihood (ML) methods to identify positively selected sites that are likely to influence the cohesiveness and mechanics of the cytoskeleton by cross-linking actinins filaments and other cytoskeleton components to create a scaffold that imparts stability and forms a bridge between the cytoskeleton and signaling pathways. We use MEGA 7 and PAML for polygenetic Analysis and positive selective Sites. Alpha-actinins from skeletal muscle as a protein factor promoting the super precipitation of actomyosin and inducing the gelation of F-actin solutions. The protein is located in Z-bands of sarcomeres in skeletal and cardiac muscles as well as near the fascia adherents of intercalated discs in cardiac muscle. In smooth muscle, it exists in cytoplasmic dense bodies and membrane-associated dense plaques. We used PAMLX to find out specific sites in these DNA sequences. These results show the location and identification of positively selected sites in the ACTININS gene family. ACTN1, ACTN2, ACTN3 and ACTN4 had (248G,250L), (234I,236N ,238P, 239K, 252F, 260E, 261Q, 314Q, 590N, 607L, 869S), (NO) and (248G, 250L) positive selective sites respectively. The aims of our work are the prediction of these sites to find the specific location of these sites in 3D structure and to replace it with suitable amino acids for the cure of obesity, muscles, cancer, and other chronic diseases.

Keywords: MEGA-7, PAMLX, maximum-likehood, actinins filaments, positively selected amino acid sites.

INTRODUCTION: The biological process of evolution is how organisms acquire the physical and physiological traits that make up a species. On the Origin of Species by Means of Natural Selection, or the Preservation of Favored Races in the Struggle for Life was Charles Darwin's 1859 theory. Evolution is a changing process. Although offspring often resemble their parents due to heredity, the structure and function of bodies alter throughout the span of generations (Smocovitis, 1992)**.** The process of change in the sequence of cellular molecules including DNA, RNA, and proteins over the course of generations is known as molecular evolution (Dietrich, 1998). New genetic variants will develop through mutation, spread through populations as a result of genetic drift or natural selection, and remain there. Mutations are irreversible, communicable alterations to a cell's or a virus's genetic make-up (DNA or RNA). The majority of mutations are single nucleotide polymorphisms, which alter just one base in the DNA sequence and cause point mutations. Duplications, insertions, deletions, inversions, and translocations are only a few of the bigger DNA segment modifications that can result from other kinds of mutations. Additionally, new genes can develop from previously non-coding DNA**.** A frame shift or stop codon mutation could result in an expanded protein that now contains a previously non-coding segment (Elena *et al.*, 2011)**.** Protein evolution is the term used to describe changes in protein composition, function, and form over time .Two proteins are said to as homologous proteins if their sequences or structural similarities show that they diverged from a single origin. In more detail, orthologs are identical proteins found in two different species. Paralogs, on the other hand, are homologous proteins that are encoded by a single species' genome. Several sequence comparisons are used to look into the evolutionary relationships of proteins. By comparing the similarities of protein sequences, one can create phylogenetic trees of proteins (Tabita *et al.*, 2007)**.**

Major cytoskeletal proteins known as alpha actinins (ACTNs) regulate crucial non-muscle processes like cytokinesis, cell adhesion, and migration in addition to mediating sarcomere function. Actinins 1-4, a family of ubiquitinated proteins, are known to be linked to actin fibers in order to preserve the cytoskeleton of cells. Actinins can be categorized as "muscle" (actinins-2 and -3) or "nonmuscle" (actinins-1 and -4) depending on where they are found (Xie *et al.*, 2020)**.** a-Actinins was first isolated from skeletal muscle as a protein factor that encouraged actomyosin superprecipitation and caused the gelation of F-actin solutions. The protein is situated close to the fascia adherens of intercalated discs in cardiac muscle 161 as well as in Z-bands of sarcomeres in skeletal and cardiac muscles. It can be found as membrane-associated dense plaques and cytoplasmic dense bodies in smooth muscle. contrary to any muscle a-actinin. The protein is localized in focal contacts, ruang membranes, and microfilament bundles with periodicity in cultured fibroblasts, according to research using immunofluorescence microscopy and microinjection with fluorescently tagged a-actinins (Tokuue *et al.*, 1991). A long-established class of actin-binding proteins is the actinins. A noteworthy case study in molecular evolution is the family of actinins, which highlights concepts like functional redundancy in duplicate genes, the evolution of protein function, and the role of natural selection in recent human evolution. proteins involved in the organization of the cytoskeleton and the control of muscle contraction (MacArthur and North, 2004)**.** A class of actin-binding proteins known as -actinins has been found in a wide variety of animals, pointing to an ancient origin. An NH2-terminal actin-binding domain, a central rod domain with four internal repeating 122-amino acid motifs, and a COOH-terminal section with two EF-hand calcium binding motifs make up the three domains of the -actinins protein structure. Given that spectrin and the four repeating motifs in -actinins are homologous, it is possible that dystrophin and the spectrin family of actin-binding cytoskeletal proteins share a common ancestor. The genes for -actinins have undergone significant evolutionary conservation across animals, especially within the NH2-terminal actin-binding region (Bogdanovich et al., 2004).

Humans have four -actinin genes, ACTN1 through ACTN4. While the skeletal muscle or sarcomeric -actinins, encoded by ACTN2 and ACTN3, have EF hands that are not calcium sensitive, ACTN1 and ACTN4 possess functional calcium-sensitive EF hands. In contrast, -actinins-3 is only expressed in rapid glycolytic skeletal muscle fibers, is absent from cardiac muscle, and has low levels of expression in the brain. In humans, -actinins-2 is found in the heart, all skeletal muscle fibers, and the brain. there function and location are presented in the table 01. ACTN1 (alpha actinins 1, Cytoskeletal muscle) is also known as F-actin cross-linking protein and Alpha-actinins cytoskeletal isoform there 3D structure is shown in the (figure 1).

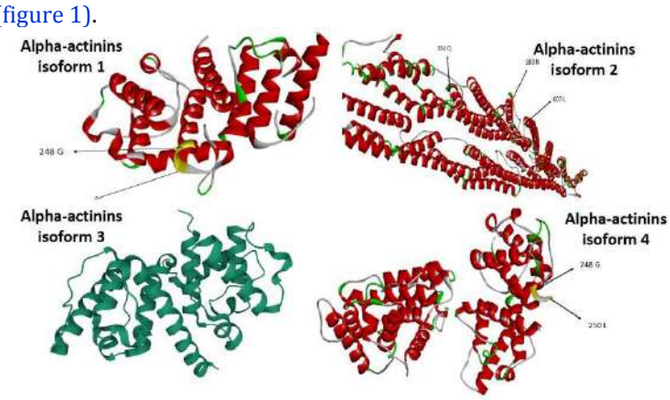

Figure 1. Structure of the actin-binding domain of human alpha-actinins 1-4

When phosphorylated, ACTN1 interacts with both and associates with the cytoplasmic domain of 1 where it promotes signaling from matrix adhesion sites and stimulates integrin-mediated cell-to-matrix adhesion.By controlling the arrangement of the actin cytoskeleton, focal adhesion, and hemi desmosome protein complexes and consequently modifying cell speed, lamellipodial

dynamics, and directed migration, ACTN1 controls the motility of keratinocytes (Hamill *et al.*, 2015).

Gene Name	Location/ production	Function	Disease	Chromosomal location
ACTININS 1	Cytoskeletal proteins, (Actinins Alpha 1) is a Protein Coding gene. This protein is located in Z-bands of sarcomeres in skeletal and cardiac muscles.	ACTN1knockdown Could induce cell cycle arrest, promote apoptosis, and inhibit EMT and cell proliferation, migration. ACTN1 knockdown inhibited subcutaneous tumor growth.	Diseases associated with ACTN1 include Bleeding Disorder, Platelet-Type, 15 and Autosomal Dominant Macro Thrombocytopenia.	14q24.1
ACTININS 2	ACTN2 gene is located at chromosome 1 (1q43) and codes a cytoskeletal protein	whereas it seems to play a role in the regulation and organization of ameloblasts.8 ACTN2 is expressed in all muscle fibers.	It has been linked to several diseases, including cardiomyopathy.	1q43
ACTININS 3	α-Actinins-3 is exclusively expressed in fast glycolytic muscle fibers.	ACTN3 is restricted to the type 2 (fast glycolytic) fibers that are responsible for forceful contraction at high velocity.	α-Actinins-3 deficiency (XX) is associated with reduced muscle strength/power and enhanced endurance performance in elite athletes and in the general population.	11q13.2
ACTININS 4	ACTN4 (gene name encoding actinins-4 protein) is located on human chromosome 19q.	Actinins-4, when expressed in cancer cell nuclei, functions as a transcriptional co-activator.	the biological roles of actinins-4 in cancer invasion.	19q13.2

Table 1: Actinins gene family Names, location, functions and its related Diseases.

RA-related synovial tissues have much higher levels of the gene for -actinins-1 (ACTN1) than OA-related synovial tissues. As a result of our assessment of the literature on -actinins (ACTNs), it is now suggested that in RA, ACTN1 may act as a "terminal effector" of intracellular signaling that has been started by tumor necrosis factor (TNF) and interleukin-1 (IL-1). Future study on ACTN1 may contribute to enhancing RA's current treatment and diagnostic approaches (Huang et al., 2014). While other (non-muscle) cells express ACTN1 with the non-muscle exon (NM), muscle cells express ACTN1 with the smooth muscle exon (SM). They describe the characterisation of a novel ACTN1 isoform in the adult rat brain in this research, in which both exons (NM + SM) are fused in a single transcript to produce a brain-specific sequence domain (BS). (RT-PCR) evidence showed that only the brain expressed the BS exon. Early postnatal stages of development revealed limited expression of the BS exon, however in adult brain it represented the major isoform of ACTN1. The cerebellum and other subcortical tissues showed only weak labeling, according to the research, while the neurons of the hippocampus, cortex, and caudate putamen displayed the highest levels of BS expression (Foley and Young, 2014). T cell migration and the stage before to migration, which involves cell attachment from suspension onto immobilized ICAM1, both required actinins1 to function. The actinins-1-targeted small interfering RNA (siRNA) was transfected into the HSB2 T cell line (Stanley *et al.*, 2008). Alpha actinin2 (ACTN2) (alpha actinins 2,) is also known as F-actin cross-linking protein or Alpha-actinins skeletal muscle isoform 2. All human skeletal muscle fibers express the -actinins-2 gene, or ACTN2as shown in the (figure 1).

In the skeletal muscle of humans, expression of -actinins-2 entirely overlaps that of -actinins-3. ACTN2 and ACTN3 are 80% and 90% similar, respectively. Additionally, -actinins-2 and -actinins-3 form heterodimers both in vitro and in vivo, demonstrating that the two skeletal muscle -actinin isoforms are structurally comparable and do not have any discernible functional distinctions. Here, it is discovered that the actin-binding protein -actinins-2, a member of the spectrin/dystrophin family, colocalizes in dendritic spines with NMDA receptors and PSD-95, a possible NMDA receptor-clustering molecule. Actinin-2 may be immunoprecipitated with NMDA receptors and PSD-95 from rat brain and binds to the cytoplasmic tails of both NR1 and NR2B subunits of the NMDA receptor through its central rod domain. It's interesting to note that Ca2+/calmodulin directly inhibits NR1--actinin binding. Therefore, NMDA receptor location and Ca2+-dependent NMDA receptor regulation may be influenced by –actinins (Wyszynski *et al.*, 1998). In the general population, cardiomyopathy (HCM), a cardiovascular ailment, affects 1 in 200 to 1 in 500 people. It is frequently distinguished by a hypertrophy left ventricle and a heart that has difficulty relaxing during diastole. Currently, it has been determined that well over 1000 mutations in a variety of sarcomeric proteins cause HCM, with more than 500 mutations found in the gene for the -cardiac myosin heavy chain (MYH7) alone. About 70% of instances of familial HCM are caused by mutations in MYH7 and cardiac myosin-binding protein C. . Numerous proteins located in the Z-disc, including the recently identified mutations in -actinins 2 (ACTN2), have been implicated in HCM. Four genes, including ACTN2, produce isoforms of -actinins, of which two (ACTN1 and ACTN4) do not belong to the muscle family. In skeletal muscle, the third gene (ACTN3) co-expresses with ACTN2, whereas only ACTN2 is expressed in cardiac muscle (Maron *et al.*, 2020).

Alpha actinin3(ACTN3): (alpha actinins), also known as F-actin cross-linking protein or Alpha-actinins skeletal muscle isoform 3 as shown in the 3D structure in (figure 1). The Z line in skeletal muscle is made up primarily of the human sarcomeric -actinins (ACTN3), which interact with a wide range of structural, signaling, and metabolic proteins to maintain the integrity of the sarcomeric layer. Type 2 (rapid glycolytic) fibers, which are in charge of vigorous contraction at high velocity, are the only ones that can express ACTN3. The ACTN3 gene carries the frequent stop codon polymorphism R577X. The absence of -actinins-3 in fast muscle fibers is caused by homozygosity for the R577X null-allele, with frequencies ranging from 1% in Africans to 18% in Caucasians. Numerous association studies have shown that the ACTN3 R577X genotype is associated with it (Oikonomou *et al.*, 2011). Alpha-actinins-3 (ACTN3) deficit is consistent with high athletic performance, according to recent cross-sectional studies.Muscle power may be correlated with an ACTN3 single nucleotide polymorphism (SNP), which may also help to explain some of the inter-individual variation in power. A premature stop codon (R577X) replaces arginine at codon 577 as a result of a C-to-T transition at position 1747 in exon 16 of the gene. Absence of ACTN3 expression is the outcome of X-allele homozygosity; there is no indication that this has anything to do with the phenotypes of muscle diseases. Caucasians have an ACTN3 deficiency rate of about 19%, demonstrating that this SNP is a widespread polymorphism in this racial group, while numerous populations around the world have an X-allele frequency of more than 15% (Delmonico *et al.*,

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2007). Energy is used more effectively by -actinins-3, whose fast fibers exhibit the metabolic and contractile characteristics of slow oxidative fibers. While this is advantageous for endurance sports, the trade-off is that the muscle is unable to produce the quick contractions required for sprinting to be successful. We suggest that the adaptive benefit of the 577X allele is also caused by the change toward more effective aerobic muscle metabolism brought on by a lack of -actinins-3. Our upcoming research will concentrate on how the ACTN3 genotype affects ageing, exercise response, and the onset and severity of muscle disease phenotype (North, 2008). Insufficient levels of -Actinins-3 (XX) are linked to improved endurance performance in both elite athletes and the general populace. Researchers have previously looked into the relationship between R577X and the loss of muscle mass and function (sarcopenia) in a number of research including aged people. However, there is some evidence that the XX genotype may be linked to a faster deterioration in muscle function. The bulk of research reveal loss of ACTN3 genotype connection with muscle features in the elderly (Seto *et al.*, 2011). Alpha actinins 4 (ACTN4) (gene name encoding actinins-4 protein) also called non-muscle alpha-actinins 4 is located on human chromosome 19q as shown in the (figure 1). ACTN4 amplification is frequently observed in patients with carcinomas of the pancreas, ovary, lung, and salivary gland, and patients with ACTN4 amplifications have worse outcomes than patients without amplification. In addition, nuclear distribution of actinins-4 is frequently observed in small cell lung, breast, and ovarian cancer. Actinins-4, when expressed in cancer cell nuclei, functions as a transcriptional co-activator. In this review, we summarize recent developments regarding the biological roles of actinins-4 in cancer invasion (Honda, 2015). An actin-binding region with two calponin homology domains is used by the widely expressed protein -Actinins-4 to crosslink actin filaments (F-actin) in a Ca2+-sensitive manner in vitro. Point mutations in the actin-binding domain of -actinins-4 lead to a hereditary, late-onset form of kidney failure. Here, we demonstrate that the enhanced actin-binding affinity of the protein is most likely the cause of the -actinins-4/F-actin clumps that have been reported in vivo in podocytes of diseased humans and mice (Weins *et al.*, 2007). One of the distinguishing features of pancreatic ductal carcinoma is an invasive growth pattern. An actin-binding protein called actinins-4 is linked to increased cell motility, invasive growth, and lymph node metastases. Actinins-4 might be crucial in the initiation and spread of pancreatic cancer (Foley and Young, 2013). Actin modification appears to be essential in the process of increasing cancer cell motility. Actinins-4 was previously found as a novel biomarker of tumour invasion and a prognostic factor for people with breast cancer. Its role in the mechanisms of cancer invasion and metastasis is still unclear, nevertheless. Actinins-4 were examined in the current study in relation to the motility and colorectal cancer cells (Taraslia *et al.*, 2018). In comparison to wild-type -actinins-4, mutant -actinins-4 has a stronger binding affinity for filamentous actin (F-actin). In this group of patients, there may be changes in how the glomerular podocytes' actin cytoskeleton is regulated. Our findings have significance for comprehending the function of the cytoskeleton in the pathophysiology of kidney disease and may help to clarify the hereditary basis of kidney damage susceptibility (Kaplan *et al.*, 2000).

OBJECTIVES: The objectives of this study were as (1) Alignment and refinement of orthologous sequences of ACTININS gene family. Prediction of positive evolveng Residues in actinins gene family.

MATERIAL AND METHODS: **Primates sequences:** Orthologous sequences of primates actinins related protein 2/3 complex gene family obtain from HGNC/NCBI using blast for obtaining only primates' sequences as shown in the (figure 2). Genomes are highly dynamic entities that evolve rapidly and non-uniformly through time; the genomes of primates are no exception. As genome-wide data and new technologies have become available, structural variation and copy-number differences have emerged as another important aspect of primate genetic variation with limited exceptions such as the gibbon there are relatively few cytological differences among primate chromosomes. Most of the cytogenetic differences between humans and apes have now been well characterized and most resolved at the molecular level. However, the majority of structural changes smaller than a few mega base pairs preclude detection by standard cytogenetic approaches. With the sequencing of the non-human primate genomes, the extent of this sub microscopic variation became more evident.

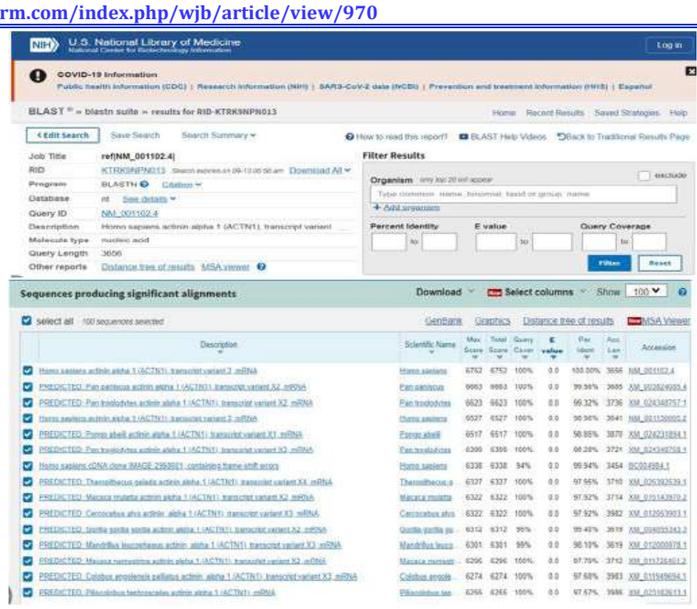

Figure 2: Primate sequences from (NCBI).

A comparison of human and chimpanzee genomes estimated more than ~90 Mb of DNA affected by insertion, deletion, duplication, and inversion (being ~40–45 Mb in each lineage). Although single-base pair changes are far more numerous, structural variants have been estimated to affect 3–4 times the number of base pairs between human and great ape (i.e., 90 million base pairs of structural variation versus 30 million base pairs of single nucleotide difference between human and chimpanzee. A recent report using macaque as an out-group has suggested that it is the chimpanzee lineage with an excess of positive selection compared to humans. The unique attributes of the primate order, and more specifically that of human, may be explained either as a consequence of key amino-acid changes within the coding sequences of a subset of critical genes or as a result of dramatic changes in how genes are regulated both temporally and spatially. Genome-wide analyses have provided traction for both of these views, although not with equal levels of support. A critical component of these analyses has been the construction of rigorous multiple-sequence alignments among primate genes.

Basic local alignment tool (BLAST): The human arp2/3 complex gene sequence was used as quarry of the BLAST of 13 organisms' sequence were download in seqdump.txt form the seqdump.txt files are change through extinction txt to MEGA format. The Basic Local Alignment Search Tool (BLAST) finds regions of local similarity between protein or nucleotide sequences. The program compares nucleotide or protein sequences to sequence in a database and calculates the statistical significance of the matches. BLAST is an acronym for Basic Local Alignment Search Tool and refers to a suite of programs used to generate alignments between a nucleotide or protein sequence, referred to as a "query" and nucleotide or protein sequences within a database, referred to as "subject" sequences as shown in the tables 2-5. The original BLAST program used a protein "query" sequence to scan a protein sequence database. A version operating on nucleotide query" sequences and a nucleotide sequence database soon followed. The introduction of an intermediate layer in which nucleotide sequences are translated into their corresponding protein sequences according to a specified genetic code allows cross-comparisons between nucleotide and protein sequences.

Molecular evolutionary genetic analysis (MEGA7): The molecular evolutionary genetic analysis (MEGA) software is used for phylogenetic tree. The evolutionary distance is computed using the neighbour joining method. MEGA7 for refining and alignment of sequence alignment through CLUSTAL W for the multiple sequence alignment to find similarities among sequence. The other purpose of MEGA 7 is to check phylogenetic tree for neighbour's species. By alignment on MEGA 7 we will remove the gaps and blank spaces from DNA sequence of the species (Kumar *et al.*, 2018).

Construction of phylogenetic tree: You can construct different kinds of trees such as ML, Neighbor-Joining, Maximum Parsimony, and so on depending upon your data. Go to the main window of MEGA7. Click Phylogeny --> Construct/Test Maximum Likelihood Tree. Select the converted file (mega) click Open. A new window will appear 'Analysis Parameters'. Here you can set different values such as bootstrapping value, substitution model, and so on. It is recommended to test phylogeny by bootstrapping for 500-1000

ACC ID	*Species*	Sequence Length	Identity %	Function annotation
NM_001102.4	*Homo sapiens*	6752	100	Homo sapiens actinins alpha 1 (ACTN1), transcript variant 2, mRNA
XM_023182613.1	*Piliocolobus tep*	6255	97.57	PREDICTED: Piliocolobus tephrosceles actinins alpha 1 (ACTN1), mRNA
XM_033187629.1	*Trachypithecus francoisi*	4837	98.03	PREDICTED: Trachypithecus francoisi actinins alpha 1 (ACTN1), transcript variant X3, mRNA
XM_035260806.1	*Aotus nancymaae*	5884	96.23	PREDICTED: Aotus nancymaae actinins alpha 1 (ACTN1), transcript variant X2, mRNA
XM_003275155.3	*Sapajus apella*	5777	96.70	PREDICTED: Sapajus apella actinins alpha 1 (ACTN1), transcript variant X2, mRNA
XM_012781152.2	*Microcebus murin*	5354	94.40	PREDICTED: Microcebus murinus actinins alpha 1 (ACTN1), transcript variant X2, mRNA
XM_003797367.3	*Otolemur garnettii*	5068	98.34	PREDICTED: Otolemur garnettii actinins alpha 1 (ACTN1), transcript variant X1, mRNA
XM_003824087.4	*Pan paniscus*	6668	99.66	PREDICTED: Pan paniscus actinins alpha 1 (ACTN1), transcript variant X1, mRNA
XM_016926257.2	*Pan troglodytes*	6628	99.24	PREDICTED: Pan troglodytes actinins alpha 1 (ACTN1), transcript variant X1, mRNA
XM_004055345.3	*Gorilla gorilla gori*	6633	99.43	PREDICTED: Gorilla gorilla actinins alpha 1 (ACTN1), transcript variant X1, mRNA
XM_030812506.1	*Nomascus leuco*	6441	98.47	PREDICTED: Nomascus leucogenys actinins alpha 1 (ACTN1), transcript variant X1, mRNA
XM_003901964.4	*Papio Anubis*	4584	98.32	PREDICTED: Papio Anubis actinins alpha 1 (ACTN1), transcript variant X1, mRNA
XM_032134793.2	*Hylobates moloch*	6094	98.24	PREDICTED: Hylobates Moloch actinins alpha 1 (ACTN1), transcript variant X1, mRNA
XM_015143969.2	*Macaca mulatta*	6325	98.24	PREDICTED: Macaca mulatta actinins alpha 1 (ACTN1), transcript variant X1, mRNA

Table 2: Primate sequences length, identity and function annotation of ACTN1.

ACC ID	*Species*	Sequence Length	Identity %	Function annotation
NM_001103.4	*Homo sapiens*	4872	100	Homo sapiens actinins alpha 2 (ACTN2), transcript variant 1, mRNA
XM_016941480.1	*Pan troglodytes*	4815	98.89	PREDICTED: Pan troglodytes actinins alpha 2 (ACTN2), transcript variant X1, mRNA
XM_034949512.1	*Pan paniscus*	4861	98.35	PREDICTED: Pan paniscus actinins alpha 2 (ACTN2), transcript variant X1, mRNA
XM_030812633.1	*Nomascus leucogenys*	4234	97.81	PREDICTED: Nomascus leucogenys actinins alpha 2 (ACTN2), transcript variant X1, mRNA
XM_032136813.1	*Hylobates moloch*	4246	97.62	PREDICTED: Hylobates moloch actinins alpha 2 (ACTN2), transcript variant X1, mRNA
XM_011729431.1	*Macaca nemestrina*	4233	96.12	PREDICTED: Macaca nemestrina actinins alpha 2 (ACTN2), transcript variant X1, mRNA
XM_012036235.1	*Cercocebus atys*	4245	96.06	PREDICTED: Cercocebus atys actinins, alpha 2 (ACTN2), transcript variant X1, mRNA
XM_005539706.2	*Macaca fascicularis*	4098	96.25	PREDICTED: Macaca fascicularis actinins alpha 2 (ACTN2), transcript variant X1, mRNA
XM_004028645.3	*Gorilla gorilla*	3481	99.16	PREDICTED: Gorilla gorilla actinins alpha 2 (ACTN2), transcript variant X1, mRNA
XM_032242265.1	*Sapajus apella*	4654	93.94	PREDICTED: Sapajus apella actinins alpha 2 (ACTN2), transcript variant X1, mRNA
XM_003893720.5	*Papio anubis*	3519	96.75	REDICTED: Papio Anubis actinins alpha 2 (ACTN2), transcript variant X1, mRNA
XM_007989887.2	*Chlorocebus sabaeus*	3481	96.09	PREDICTED: Chlorocebus sabaeus actinins alpha 2 (ACTN2), transcript variant X1, mRNA
XM_023216194.2	*Piliocolobus tephroscles*	3310	97.22	PREDICTED: Piliocolobus tephrosceles actinins alpha 2 (ACTN2), transcript variant X1, mRNA
XM_035281481.1	*Callithrix jacchus*	3316	94.28	PREDICTED: Callithrix jacchus actinins alpha 2 (ACTN2), transcript variant X1, mRNA

Table 3: Primate sequences length, Identity and function annotation of ACTN2.

ACC ID	Species	Sequence Length	Identity %	Function annotation
NM_001104.4	*Homo sapiens*	2882	100	Homo sapiens actinins alpha 3 (ACTN3), transcript variant 1, coding, mRNA
XM_016921306.2	*Pan troglodytes*	2939	99.13	PREDICTED: Pan troglodytes actinins alpha 3 (ACTN3), mRNA
XM_003828673.3	*Pan paniscus*	2939	99.10	PREDICTED: Pan paniscus actinins alpha 3 (ACTN3), mRNA
XM_004051591.2	*Gorilla gorilla*	2939	98.92	PREDICTED: Gorilla gorilla actinins alpha 3 (ACTN3), mRNA
XM_001109839.3	*Macaca mulatta*	2939	97.25	PREDICTED: Macaca mulatta actinins alpha 3 (ACTN3), mRNA
XM_007973971.2	*Chlorocebus sabaeus*	2939	97.25	PREDICTED: Chlorocebus sabaeus actinins alpha 3 (ACTN3), transcript variant X1, mRNA
XM_012041564.1	*Cercocebus atys*	3482	97.18	PREDICTED: Cercocebus atys actinins, alpha 3 (ACTN3), mRNA
XM_003909508.5	*Papio anubis*	2939	97.15	PREDICTED: Papio anubis actinins alpha 3 (ACTN3), mRNA
XM_023186108.1	*Piliocolobus tephroceles*	2948	96.57	PREDICTED: Piliocolobus tephrosceles actinins alpha 3 (ACTN3), mRNA
XM_010366410.2	*Rhinopithecus roxellana*	2943	96.49	PREDICTED: Rhinopithecus roxellana actinins alpha 3 (ACTN3), mRNA
XM_002807373.5	*Callithrix jacchus*	3469	95.28	PREDICTED: Callithrix jacchus actinins alpha 3 (ACTN3), mRNA
XM_032282730.1	*Sapajus apella*	2942	95.17	PREDICTED: Sapajus apella actinins alpha 3 (ACTN3), mRNA
XM_017537045.1	*Cebus imitator*	2947	94.83	PREDICTED: Cebus imitator actinins alpha 3 (ACTN3), mRNA
XM_012665275.1	*Propithecus coquereli*	2914	93.50	PREDICTED: Propithecus coquereli actinins, alpha 3 (ACTN3), transcript variant X1, mRNA

Table 4: Primate sequences length, Identity and function annotation of ACTN3.

ACC ID	*Species*	Sequence Length	Identity %	Function annotation
NM_001104.4	*Homo sapiens*	2882	100	Homo sapiens actinins alpha 3 (ACTN3), transcript variant 1, coding, mRNA
XM_016921306.2	*Pan troglodytes*	2939	99.13	PREDICTED: Pan troglodytes actinins alpha 3 (ACTN3), mRNA
XM_003828673.3	*Pan paniscus*	2939	99.10	PREDICTED: Pan paniscus actinins alpha 3 (ACTN3), mRNA
XM_004051591.2	*Gorilla gorilla*	2939	98.92	PREDICTED: Gorilla gorilla actinins alpha 3 (ACTN3), mRNA
XM_001109839.3	*Macaca mulatta*	2939	97.25	PREDICTED: Macaca mulatta actinins alpha 3 (ACTN3), mRNA
XM_007973971.2	*Chlorocebus sabaeus*	2939	97.25	PREDICTED: Chlorocebus sabaeus actinins alpha 3 (ACTN3), transcript variant X1, mRNA
XM_012041564.1	*Cercocebus atys*	3482	97.18	PREDICTED: Cercocebus atys actinins, alpha 3 (ACTN3), mRNA
XM_003909508.5	*Papio anubis*	2939	97.15	PREDICTED: Papio anubis actinins alpha 3 (ACTN3), mRNA
XM_023186108.1	*Piliocolobus tephroceles*	2948	96.57	PREDICTED: Piliocolobus tephrosceles actinins alpha 3 (ACTN3), mRNA
XM_010366410.2	*Rhinopithecus roxellana*	2943	96.49	PREDICTED: Rhinopithecus roxellana actinins alpha 3 (ACTN3), mRNA
XM_002807373.5	*Callithrix jacchus*	3469	95.28	PREDICTED: Callithrix jacchus actinins alpha 3 (ACTN3), mRNA
XM_032282730.1	*Sapajus apella*	2942	95.17	PREDICTED: Sapajus apella actinins alpha 3 (ACTN3), mRNA
XM_017537045.1	*Cebus imitator*	2947	94.83	PREDICTED: Cebus imitator actinins alpha 3 (ACTN3), mRNA
XM_012665275.1	*Propithecus coquereli*	2914	93.50	PREDICTED: Propithecus coquereli actinins, alpha 3 (ACTN3), transcript variant X1, mRNA

Table 5: Primate sequences length, identity and function annotation of ACTN4.

times as shown in figure 3. For ACTN1,2,3,4 Respectively. Additionally, select the substitution model appropriately. You can use other software such as Prottest3 to find an appropriate model for your data.

Phylogenetic analysis by maximum liklihood (PAML) for positve prediction: We used PAMLX software, the PAMLX is used for finding the positive sites in DNA sequence, in the calculation of Phylogenetic analysis by maximum likelihood (PAML) include the programmes such as baseml, codeml, evolver, chi2 etc. PAML is a package of programs for phylogenetic analyses of DNA or protein sequences using maximum likelihood. It may be used to estimate parameters and test hypotheses to study the evolutionary process, when you have reconstructed trees using other programs such as PAUP*, PHYLIP, MOLPHY, PhyML, RaxML. We use the PAML

software for the prediction of positive selective sites.

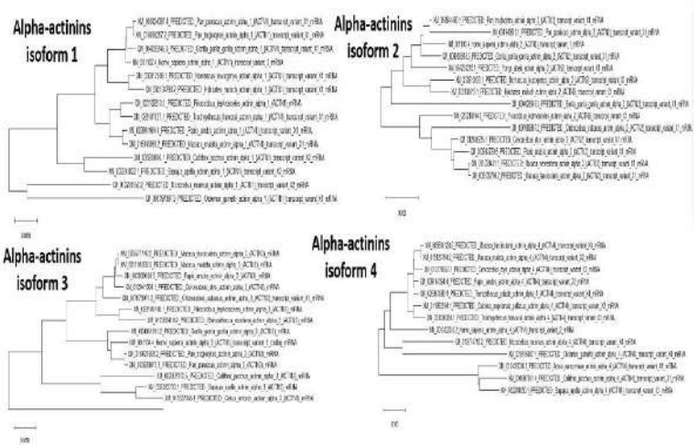

Figure 3: Phylogenetic analysis of alpha actinin gene 1-4.

RESULTS AND DISCUSSION: Positive selection is likely to play a key role in the function of the protein. we used codon-based substitution models and maximum-likelihood (ML) methods to identify positively selected sites that are likely to be influences the cohesiveness and mechanics of the cytoskeleton by cross-linking actinins filaments and other cytoskeleton components to create a scaffold that imparts stability and forms a bridge between the cytoskeleton and signalling pathways. We identify the orthologous sequences of actins gene family. We examined the molecular evolution in the primate sequences of gene (ACTN1, ACTN2, ACTN3, and ACTN4) of actinins. For each gene, a phylogenetic tree based on amino acid sequences was used as the input in the analysis of molecular evolution Maximum-likelihood estimates of ω based on a single ratio across all Branches, respectively. However, Have Positive sites in ACTN1 has 248 G ,250 L positive selective sites. ACTN2 has 234I,236N ,238P, 239K, 252F, 260E, 261Q, 314Q, 590N, 607L, 869S. ACTN3 has NO positive selective sites. ACTN4 has 248G, 250L positive selective sites were predicted on the basis "Naive Empirical Bayes" (NEB) and "Bayes Empirical Bayes" (BEB) analysis as shown in table 6.

Genes	Site Model	ln L	X2	P value	ω in M8	Positive sites
ACTN 1	M 7 M 8	-3099.449219 -3098.868440	-0.00010884755	<0.028 M8 np= 28 M7 np= 26 df=2	1.59393	248G 250L
ACTN 2	M 7 M 8	-5134.560650 -5122.676695	-0.027569256 24	<0.004 M8 np= 30 M7 np= 28 df=2	2.40391	234 I,236 N 238 P, 239K, 252F, 260 E, 261Q, 314Q, 590N, 607L, 869 S.
ACTN 3	M 7 M 8	-5977.949355 -5977.949361	-6.022132415	<0.002 M8 np= 32 M7 np= 30 df=2	1.00000	NO POSITIVE SITES
ACTN 4	M 7 M 8	-6730.669821 -6730.201418	-0.0003259953	<0.029 M8 np= 28 M7 np= 26 df=2	1.00000	248G 250L

Table 6: Likelihood ratio tests of site-specific models on ACTININS gene family performed in PAML.

Alpha actnin1 (ACTN1): Two Positive Selective Sites on ACTN1 are 248G and 250L The transduction of mouse fatal liver-derived megakaryocytes with disease-associated ACTN1 variants caused a disorganized actin-based cytoskeleton in megakaryocytes, resulting in the production of abnormally large pro platelet tips that were reduced in number (Kunishima *et al.*, 2013) changed the position 255 E which is close to our positive selective sites 248G and 250L respectively. Our research sheds light on the causes of macrothrombocytopenia (CMTP).

Alpha actinin2 (ACTN2): 234I, 236N, 238P, 239K, 252F, 260 E, 261Q, 314Q, 59 N, 607L, and 869S are all present in ACTN2. The hypertrophic cardiomyopathy-causing site 583E, which is close to our positive sites 590N and 607L, was changed in 2010 by Christine Chiu et al. [58]. The gene location 228M, which is close to our positive selective sites and associated with familial hypertrophic cardiomyopathy and juvenile atrial arrhythmias, was mutated (Girolami *et al.*, 2014)**.** The most prevalent hereditary cardiac illness, hypertrophic cardiomyopathy (HCM), is typically brought on by abnormalities in the sarcomere protein genes.

Alpha actinin3 (ACTN3): According to "Naive Empirical Bayes" (NEB) and "Bayes Empirical Bayes" (BEB) research, ACTN3 contains NO positive selected sites. Because ACTN3 is a stable protein and has not undergone further evolution in nature. Because ACTN3 sequences are less evolved at the protein level than they are at the DNA level, Actinins 3 do not produce any positive protein sites. As ACTN3 plays a crucial part in the cytoskeleton of the cell, if the ACTN3 protein evolves further, the cytoskeleton will change, ultimately destroying the cell's structure and impairing the cell's ability to carry out its typical function.

Alpha actnin4 (ACTN4): According to "Naive Empirical Bayes" (NEB) and "Bayes Empirical Bayes" (BEB) analysis, ACTN4 possesses 248G and 250L positive selected sites (Khurana *et al.*, 2012), which is quite close to our positive selective sites 248G and 250L and is associated with family types of focal segmental glomerulosclerosis (FSGS), a kidney condition marked by proteinuria brought on by podocyte damage.

CONCLUSIONS: According to our investigation, the ACTININS gene family exhibits favorable selective locations. In the 3D structure of the ACTININS gene family, we need to substitute an appropriate residue for residues 248G, 250L in ACTN1, 234I, 236N, 238P, 239K, 252F, 260E, 261Q, 314Q, 590N, 607L, and 869S in ACTN2, and 248G, 250L in ACTN4. These modifications could aid in the treatment of cancer, muscles, and other chronic diseases if the proper amino acid substitution is discovered. Additionally, we require enough cash to continue exploring these locations.

CONFLICT OF INTEREST: Authors have no conflict of interest

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REFERENCES: Bogdanovich, S., K. J. Perkins, T. O. Krag and T. S. Khurana, 2004. Therapeutics for duchenne muscular dystrophy: Current approaches and future directions. Journal of molecular medicine, 82: 102-115.

Delmonico, M. J., M. C. Kostek, N. A. Doldo, B. D. Hand, S. Walsh, J. M. Conway, C. R. Carignan, S. M. Roth and B. F. Hurley, 2007. Alpha-actinin-3 (actn3) r577x polymorphism influences knee extensor peak power response to strength training in older men and women. The journals of gerontology series A: Biological sciences and medical sciences, 62(2): 206-212.

Dietrich, M. R., 1998. Paradox and persuasion: Negotiating the place of molecular evolution within evolutionary biology. Journal of the history of biology: 85-111.

Elena, S. F., S. Bedhomme, P. Carrasco, J. M. Cuevas, F. De La Iglesia, G. Lafforgue, J. Lalić, À. Pròsper, N. Tromas and M. P. Zwart, 2011. The evolutionary genetics of emerging plant rna viruses. Molecular plant-microbe interactions, 24(3): 287-293.

Foley, K. S. and P. W. Young, 2013. An analysis of splicing, actin-binding properties, heterodimerization and molecular interactions of the non-muscle α-actinins. Biochemical journal, 452(3): 477-488.

Foley, K. S. and P. W. Young, 2014. The non-muscle functions of actinins: An update. Biochemical journal, 459(1): 1-13.

Girolami, F., M. Iascone, B. Tomberli, S. Bardi, M. Benelli, G. Marseglia, C. Pescucci, L. Pezzoli, M. E. Sana and C. Basso, 2014. Novel α-actinin 2 variant associated with familial hypertrophic cardiomyopathy and juvenile atrial arrhythmias: A massively parallel sequencing study. Circulation: Cardiovascular genetics, 7(6): 741-750.

Hamill, K. J., S. Hiroyasu, Z. T. Colburn, R. V. Ventrella, S. B. Hopkinson, O. Skalli and J. C. Jones, 2015. Alpha actinin-1 regulates cell-matrix adhesion organization in keratinocytes: Consequences for skin cell motility. Journal of Investigative Dermatology, 135(4): 1043-1052.

Honda, K., 2015. The biological role of actinin-4 (actn4) in malignant phenotypes of cancer. Cell & bioscience, 5(1): 1-9.

Huang, R.-Y., Q.-C. Huang and B. M. T. Burgering, 2014. Novel insight into the role of alpha-actinin-1 in rheumatoid arthritis. Discovery medicine, 17(92): 75-80.

Kaplan, J. M., S. H Kim, K. N. North, H. Rennke, L. A Correia, H.-Q. Tong, B. J. Mathis, J.-C. Rodríguez-Pérez, P. G. Allen and A. H. Beggs, 2000. Mutations in actn4, encoding α-actinin-4, cause familial focal segmental glomerulosclerosis. Nature genetics, 24(3): 251-256.

Khurana, S., S. Chakraborty, M. Lam, Y. Liu, Y.-T. Su, X. Zhao, M. A. Saleem, P. W. Mathieson, L. A. Bruggeman and H.-Y. Kao, 2012. Familial focal segmental glomerulosclerosis (fsgs)-linked α-actinin 4 (actn4) protein mutants lose ability to activate transcription by nuclear hormone receptors. Journal of biological chemistry, 287(15): 12027-12035.

Kumar, S., G. Stecher, M. Li, C. Knyaz and K. Tamura, 2018. Mega x: Molecular evolutionary genetics analysis across computing platforms. Molecular biology and evolution, 35(6): 1547.

Kunishima, S., Y. Okuno, K. Yoshida, Y. Shiraishi, M. Sanada, H. Muramatsu, K. Chiba, H. Tanaka, K. Miyazaki and M. Sakai, 2013. Actn1 mutations cause congenital macrothrombocytopenia. The American journal of human genetics, 92(3): 431-438.

MacArthur, D. G. and K. N. North, 2004. A gene for speed? The evolution and function of α-actinin-3. Bioessays, 26(7): 786-795.

Maron, B. J., S. Mackey-Bojack, E. Facile, E. Duncanson, E. J. Rowin and M. S. Maron, 2020. Hypertrophic cardiomyopathy and sudden death initially identified at autopsy. The American journal of cardiology, 127: 139-141.

North, K., 2008. Why is alpha-actinin-3 deficiency so common in the general population? The evolution of athletic performance. Twin research and human genetics, 11(4): 384-394.

Oikonomou, K. G., K. Zachou and G. N. Dalekos, 2011. Alpha-actinin: A multidisciplinary protein with important role in b-cell driven autoimmunity. Autoimmunity reviews, 10(7): 389-396.

Seto, J. T., S. Chan, N. Turner, D. G. MacArthur, J. M. Raftery, Y. D. Berman, K. G. Quinlan, G. J. Cooney, S. Head and N. Yang, 2011. The effect of α-actinin-3 deficiency on muscle aging. Experimental gerontology, 46(4): 292-302.

Smocovitis, V. B., 1992. Unifying biology: The evolutionary synthesis and evolutionary biology. Journal of the history of biology, 25(1): 1-65.

Stanley, P., A. Smith, A. McDowall, A. Nicol, D. Zicha and N. Hogg, 2008. Intermediate-affinity lfa-1 binds α-actinin-1 to control migration at the leading edge of the t cell. The EMBO journal, 27(1): 62-75.

Tabita, F. R., T. E. Hanson, H. Li, S. Satagopan, J. Singh and S. Chan, 2007. Function, structure, and evolution of the rubisco-like proteins and their rubisco homologs. Microbiology and molecular biology reviews, 71(4): 576-599.

Taraslia, V., S. Lymperi, V. Pantazopoulou, A. K. Anagnostopoulos, I. S. Papassideri, E. K. Basdra, M. Bei, E. G. Kontakiotis, G. T. Tsangaris and D. J. Stravopodis, 2018. A high-resolution proteomic landscaping of primary human dental stem cells: Identification of shed-and pdlsc-specific biomarkers. International journal of molecular sciences, 19(1): 158.

Tokuue, Y., S. Goto, M. Imamura, T. Obinata, T. Masaki and T. Endo, 1991. Transfection of chicken skeletal muscle α-actinin cdna into nonmuscle and myogenic cells: Dimerization is not essential for α-actinin to bind to microfilaments. Experimental cell research, 197(2): 158-167.

Weins, A., J. S. Schlondorff, F. Nakamura, B. M. Denker, J. H. Hartwig, T. P. Stossel and M. R. Pollak, 2007. Disease-associated mutant α-actinin-4 reveals a mechanism for regulating its f-actin-binding affinity. Proceedings of the National Academy of Sciences, 104(41): 16080-16085.

Wyszynski, M., V. Kharazia, R. Shanghvi, A. Rao, A. H. Beggs, A. M. Craig, R. Weinberg and M. Sheng, 1998. Differential regional expression and ultrastructural localization of α-actinin-2, a putative nmda receptor-anchoring protein, in rat brain. Journal of neuroscience, 18(4): 1383-1392.

Xie, G.-f., L.-d. Zhao, Q. Chen, D.-x. Tang, Q.-y. Chen, H.-f. Lu, J.-r. Cai and Z. Chen, 2020. High actn1 is associated with poor prognosis, and actn1 silencing suppresses cell proliferation and metastasis in oral squamous cell carcinoma. Drug Design, Development and Therapy: 1717-1727.