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The Importance of ACTN3 R577X Polymorphism in Athletic Performance and Modeling of R577X Mutant Type and Wild Type ACTN3 Protein by Bioinformatics Analysis

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Abstract: In studies conducted on the relationship between ACTN3 R577X polymorphism and athletic performance, it is known that athletic performance is polygenic, but the most sensitive relationship is provided by the ACTN3 gene. Considering the results, the conformational difference is much less (<0.05 A) between wild type and R577X. R577X has fewer amino acids (324 amino acids) than the wild type. It has been observed that the subunits of the R577X model do not have 4 long and 5 short alpha-helical curves. According to the analysis results of physico-chemistry properties, each model is very close to alipatich endex, but the R577X mutant type is higher than the wild type. Each model contains a large number of hydrophilic amino acids. R577 was found to be more hydrophilic than wild type. Each model has more likely amino acids versus negative amino acids. However, it considers the R577X mutant type more likely than the wild type. The purpose of this study is; explaining physico and chemistry properties with 3D homology between the wild type of ACTN gene and the R577X mutation region and contributed to future studies on this subject by making homology models. Uniprot, Swiss Model and Chimera were used as bioinformatics transition. Our results showed that there was no significant change in the molecular structure, in which case the function of the protein was not impaired. However, athletes with this mutation at the end of intense muscle performances are more susceptible to muscle damage. R577X mutant and wild-type protein of ACTN3 the physico-chemical properties of three-dimensional homology model was performed for the first time in Turkey.
Keywords: ACTN3, Alpha actin3 genleri, R577X, rs1815739, 3D homoloji

ACTN3 R577X Polimorfizminin Atletik Performasyondaki Önemi ve R577X Mutant Tip ve Wild Tip ACTN3 Proteininin Biyoinformatik Analizi ile Modellemesi

Özet: ACTN3 R577X polimorfizmi ile atletik performans arasındaki ilişki üzerine yapılan çalışmalarda atletik performansın poligenik olduğu bilinmekle birlikte en hassas ilişkinin ACTN3 geni tarafından sağlandığı belirtilmektedir. Sonuçlar göz önüne alındığında, wild tip ve R577X arasında konformasyonel fark çok daha azdır (<0,05 A). R577X, wild tipten daha az amino aside (324 amino asit) sahiptir. R577X modelinin alt birimlerinin 4 uzun ve 5 kısa alfa-sarmal eğriye sahip olmadığı görülmüştür. Fiziko-kimya özelliklerinin analiz sonuçlarına göre her model alifatik indekse çok yakındır ancak R577X mutant tipi, wild tipten daha yüksektir. Her model çok sayıda hidrofilik amino asit içerir. R577 mutant tipinin wild tipten daha hidrofilik olduğu bulundu. Her model, negatif amino asitlere karşı daha olası amino asitlere sahiptir. Bununla birlikte, R577X mutant tipinin, wild tipten daha olası olduğu düşünülmektedir. Bu çalışmanın amacı; Wild tip ACTN geni ile R577X mutasyon bölgesi arasındaki fiziko ve kimya özelliklerini 3 boyutlu homoloji ile açıklamak ve homoloji modelleri yaparak bu konuda ileride yapılacak çalışmalara katkı sağlamaktır. Biyoinformatik bağlantı olarak Uniprot, Swiss Model ve Chimera kullanılmıştır. Sonuçlarımız, moleküler yapıda önemli bir değişiklik olmadığını, bu durumda proteinin işlevinin bozulmadığını gösterdi. Ancak yoğun kas performanslarının sonunda bu mutasyona sahip sporcular kas hasarına daha duyarlıdır. ACTN3'ün R577X mutant ve wild tip proteininin, Türkiye'de ilk kez üç boyutlu homoloji modeli ile fiziko-kimyasal özellikleri gösterildi.
Anahtar Kelimeler: ACTN3, Alpha actin3 genes, R577X, rs1815739, 3D homology

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INTRODUCTION

Alpha actinin 3 (ACTN3) is a muscle protein encoded by the ACTN3 gene and an important structural component of the Z disc. It has been shown in studies that this protein is an important marker in elite sportive performance (Oliveira et al., 2018; Şanlısoy et al., 2011). Sportive performance is based on a combination of an individual's inherent genetic abilities and environmental factors such as appropriate training exercise and nutrition (Kaman et al. 2017; Oliveira et al., 2018). In recent years, studies in the field of sports genetics by identifying the genes that affect sports performance and elucidating their mechanisms of action have gained importance with the increasing economy of sports (Ulucan et al., 2016; Mutlucan et al. 2017). The ACTN3 gene, which consists of 22 exons, is located at the 11q13.1. ACTN3 protein is 103241 Da, consisting of 901 amino acids, responsible for fast and strong contractions during sports activities requiring muscle power, serving in glycolytic type and type-IIX muscle strands, binding actin fibrils in muscle contraction, as well as a protein that has active roles in intracellular signal transduction (Kaman et al. 2017; Kikuchi et al. 2017). The high-resolution three-dimensional structure of the ACTN3 protein was published by Franzot et al. in 2005 as a result of many years of X-ray studies. ACTN3 protein deficiency occurs in individuals with rs1815739 polymorphism (R577X). This deficiency does not cause any serious muscle disease, but results in differences in muscle strength function (Ulucan et al., 2016; Mutlucan et al. 2017; Belli et al. 2017; Papadimitriou et al., 2018). In addition, same cases indicated the positive association between the presence of the 577R allele and the capacity to perform high power muscle contractions (Clarskon et al., 2005; Delmonico et al., 2007). In the study that Seto et al. in 2013 has shown that ACTN3 deficiency in mouse and human muscle cells increases calcineurin activity and their adaptation to endurance training is faster. Sprinters, weightlifters and swimmers statistically important differences in genotypes were only indicated in sprinters (Cięszczyk et al., 2011). The number of studies on the relationship between ACTN3 R577X polymorphism and athletic performance is quite high in the world. The general results of these analysis studies are that athletic performance is polygenic and the most susceptibility relationship is provided by the ACTN3 gene (Massidda et al., 2015). Structural bioinformatics, which are protein structure studies, is one of the subtitles of bioinformatics and is the whole of the studies performed to predict, develop and define molecular structures (Dill, 1990). Proteins are sequences including many physiological processes directly that molecules found in all organisms and made up of unique amino acid. The 3D structure of a protein can define to the biological significance of that protein and its work within a process. Mutations can change molecular structure then it can effect function of proteins. Any change in the protein sequence (deletion, insertion or mutation) can trigger to reconformation change a specific or a whole structure by disrupting the balance of interaction forces within the protein. All these changes in the protein structures are analyzed and evalueted using bioinformatics tools at the present time. Homology based modeling studies are one of the important techniques used in the three-dimensional structures of proteins. Evaluation of the physico-chemical properties and homology models of proteins is of great importance in predicting the structure of proteins. Knowing the structure of a protein's mutant or wild type is a guide in understanding protein-protein interactions, protein-ligand interactions, and docking studies (Oflaz, 2017). The physico-chemical properties and structure analysis of proteins contribute to the understanding of the functions of proteins, the development of experimental studies, the solve of the metabolism / mechanism of the genetic disease, the clarify of protein mechanism (Jones, 1999).

MATERIALS and METHOD

Databases and homology modelling

The sequence of the ACTN3 Protein was obtained from the UniProtKB database (uniprot refferans, code: Q08043) and based on this sequence the sequence of the R577X mutant type was arranged. Three dimensional models of ACTN3 Protein wild and R577X mutant types were created using Swiss-Model Bioinformatics Portal (Swiss Model, 2021).

Bioinformatic analysis of homology models

The designed homology models were analyzed and visualized using UCSF Chimera (1.13) program (UCSF Chimera, 2021). The physico-chemical properties of the wild type and R577X mutant type of the ACTN3 protein were analyzed using the ExPASy-ProtParam Portal (ExPASy, 2021). Amino acid number, molecular weight, theoretical pI value, amino acid composition, negatively and positively charged amino acid numbers, aliphatic index and hydrophobicity value were analyzed for each model.

RESULTS

Comparison of homology models

Homology models are overlapped. It was observed that the conformational alteration in the overlapping regions was very small (<0.05 Å) with the ribbon display. The R577X mutant type is 324 fewer amino acids than the wild type, lost amino acids are colored red in the Figure 1. It was observed that the subunits of the R577X model did not have 4 long and 5 short alpha-helix folds (Figure 1).

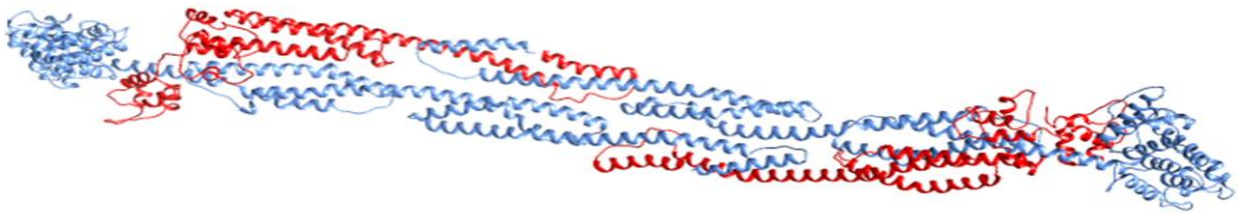


Figure 1. R577X mutant type is colored blue, amino acids lost by deletion are colored red (Orijinal figure).

Physico-chemical analysis

According to the physico-chemical properties of wild and R577X mutant type of ACTN3 protein, amino acid number, molecular weight, theoretical pI value, amino acid composition, negatively and positively charged amino acid numbers, aliphatic index and hydrophobicity value are given in Table 1.

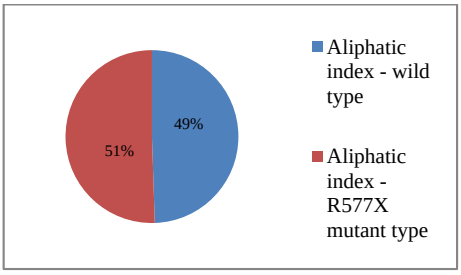
Table 1. ProtParam Analysis results

	Grand average of hydropathicity	Aliphatic index	Total number of aminoacids	Molecular weight	Negative charged aminoacids	Possitive charged aminoacids
Wild Type	-0.507	84.84	901	103241.29	141 (%56)	113 (%44)
R577X	-0.551	86.64	577	66630.90	95 (%55)	79 (%45)

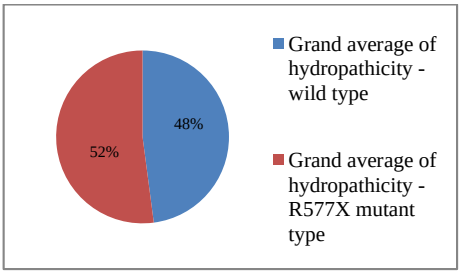
Bioinformatics analysis of ACTN3 wild type and R577X showed that:

- According to the results of the physico-chemical analysis, the aliphatic indices of both models are quite close to each other.
- The aliphatic index of R577X mutant type is higher than the wild type (Graphic 1).
- Both models are rich in hydrophilic amino acids.
- The hydropathy index showed that R577X mutant type is more hydrophilic (Graphic 2).

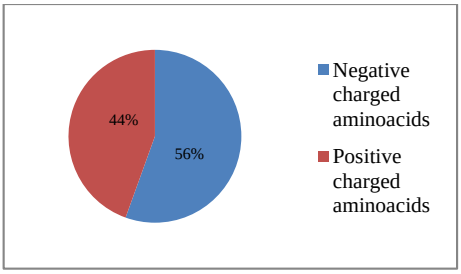
- The number of negatively charged amino acids in both models is greater than the number of positively charged amino acids, both models are rich in negatively charged amino acids (Graphic 3, Graphic 4).
- When compared in proportion showed that R577X mutant type is more positive than the wild type.
- When the ratios of negative amino acids to positive amino acids were compared, it was seen that the R577X mutant type contains more positive amino acids than the wild type.



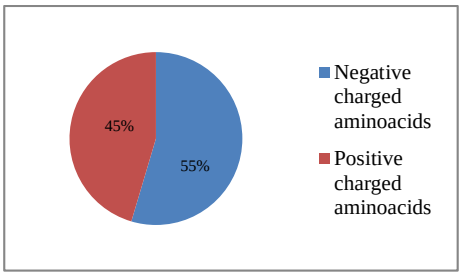
Graphic 1. Aliphatic index ratio of wild type and R577X mutant type.



Graphic 2. Grand average of hydropathicity ratio of wild type and R577X.



Graphic 3. Negative and positive charged aminoacids ratio of wild type.



Graphic 4. Negative and positive charged aminoacids ratio of R577X mutant type.

DISCUSSION

Actin can be divided into three main groups as alpha, beta, and gamma. They are abundant in eukaryotic cells and also play a role in the cytoskeleton and motility. While beta actin and gamma actin are found in all cells, alpha actin is normally limited to smooth muscle cells (Ruan and Lai, 2007).

Alpha actinins are a family of dystrophin-related actin-binding proteins. It has structural and regulatory roles in cytoskeleton organization and muscle contraction (Yang et al., 2003).

Two skeletal muscle isoforms of alpha actinin (ACTN2 and ACTN3) are the main structural components of the Z line required to contain thin filaments in the muscle. In humans, ACTN2 was expressed in all muscle fibers, whereas expression of ACTN3 was limited to a subset of type 2 fibrils that showed rapid and sudden traction. Alpha-actin-3, whose expiration is limited in fast glycolytic fibrils in skeletal muscle, is the most specialized of the four mammalian alpha actinins. Alpha actinin 3 enables fast-pulling fibrils to produce larger amounts of motion at higher speeds (Del Coso et al., 2019).

The ACTN3 gene is responsible for the production of alpha-actin-3 and located in the 11q13-q14 region. As a result of the C1729T mutation at exon 16 of the ACTN3 gene, the stop codon is formed and the codon at position 577 produces the arginine amino acid converts to the stop codon (R577X). ACTN3 gene and its effect on athletic performance characteristics are among the current research topics. In recent years, with the development of molecular techniques, the investigation of various genetic characteristics of athletes has gained momentum and the effect of alpha actinin 3 (ACTN3) gene on athletic performance has been

studied (Şanlısoy et al., 2011; Ulucan et al., 2016; Mutlucan et al., 2017; Oliveira et al., 2018; Bulgay et al., 2020).

Between the ACTN3 genotypes and the association performance of elite athletes, if α-actin3 type II had a significant effect on muscle fibers. It has been reported that it is possible to predict differences in skeletal muscle function for ACTN3 among individuals with different genotypes (R577X). It has been reported that the presence of the 577R allele in speed / strength athletes is consistent with the rapid contraction of skeletal muscle fibers of α-actin 3 (Şanlısoy et al., 2011).

It is mentioned ACTN3 and ACE genes, which are important genetic variables responsible for sportive performance, are thought to be effective in screening the skills of candidates to become athletes, determining the level of muscle damage, determining sports branches, in-branch guidance, and using appropriate training programs (Bulgay et al., 2020).

Structural bioinformatics, which is protein structure studies, is a collection of studies conducted to predict, develop and define molecular structures as one of the subtitles of bioinformatics (Dill, 1990).

The physico-chemical properties and structure analysis of proteins contribute to the understanding of the functions of proteins, the development of experimental studies, the solution of the metabolism / mechanism of genetic disease, and the clarification of the protein mechanism (Jones, 1999).

CONCLUSION

According to our results, R577X has fewer amino acids (324 amino acids) than wild type. It has been observed that the subunits of the R577X model do not have 4 long and 5 short alpha-helical curves. According to our analysis results of physico-chemistry properties, each model is very close to alipatich endex, but the R577X mutant type is higher than the wild type. Each model contains a large number of hydrophilic amino acids. R577 has been found to be more hydrophilic than wild type. Each model has more likely amino acids versus negative amino acids. However, it considers the R577X mutant type more likely than the wild type. Our study results showed that there was no significant change in the molecular structure other than the deletion regions, in which case the function of the protein was not damaged. We think that the presence of long and short alpha-helix folding of the deletion regions may enable the protein to work more sensitive to muscle damage. Regarding the place we call outside the erasing areas; this is because the last 80 amino acids in the R577X mutation are deleted and end with a stop codon. Therefore, the last 80 amino acids of wild type are not present in the mutant type. The location we call outside the deletion region is the first 174 amino acids of both models. Our results showed that there was no significant change in the molecular structure, in which case the function of the protein was not impaired. However, athletes with this mutation are more susceptible to muscle damage at the end of intense muscle performances (Belli et al. 2017; Bulgay et al., 2020).

The physico-chemical properties of Wild type and R577X mutation of ACTN3 protein are explained by 3-dimensional homology. Since it is the first time that homology models are made in Turkey, we think we will contribute to future studies on this subject R577X mutant and wild-type protein of ACTN3.

Conflict of Interest: The authors declare that they have no conflict of interest.

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