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Case Report

Limb-girdle muscular dystrophy: A rare clinical case report

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ABSTRACT

The TTN gene encodes the largest human protein (OMIM 188840). It is known pathogenic variants in this gene can lead to cardiomyopathy and muscle diseases. Mutations in this gene can also occur during the congenital period. According to OMIM data, the diseases that can be identified by the mutation type in our study include muscular dystrophy, limb-girdle, autosomal recessive 10, and Salih myopathy. In this study, the TTN NM_001267550.2:c.32676del frameshift variant was identified as homozygous in the patient. This homozygous mutation, which has been retained in the patient, is manifested in the family as carrier status. The patient's family is asymptomatic. In this case report, a 21-month-old male patient applied to the pediatric neurology clinic with complaints of delayed motor-milestones and difficulty in walking. Clinical exome sequencing, next-generation sequencing, and Sanger analyses were used as methods in the analysis. Although the mutation observed in the patient is listed as likely pathogenic in ClinVar, we did not encounter this exact mutation in the literature.

Keywords: Autosomal recessive, frameshift, likely pathogenic, muscular dystrophy, titin.

Myopathies are a group of diseases characterized primarily by muscle weakness. Myopathies are classified under the main heading of "core myopathy," which includes subcategories such as "central core disease," "multiminicore disease (MmD)," "dusty core disease," and "core-rod myopathy."

In the realm of myopathies, which have been subdivided into numerous subcategories, as per the OMIM database, limb-girdle, also referred to as Salih myopathy and autosomal recessive 10, is classified within the categorization of "core myopathy" under the subheading of "multiminicore disease." This ailment has found its place in the literature as a myopathy associated with the TTN gene.[1]

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TTN (Online Mendelian Inheritance in Man database [OMIM] #188840) is a large gene that is located on long arm of chromosome 2 and encodes 4200 kDa large protein titin.[2] Titin (and its isomers) is the biggest highly elasticated protein found in nature. Its primary function is to stabilize thick filaments at the center of the sarcomere of skeletal and cardiac muscle. Its N terminal binds to the Z-line and C terminal to the M-line of the sarcomere, thus centers thick filaments between thin filaments, prevents overstretching, and provide recoiling to the resting state.[3,4] Titin-related myopathies are heterogenous group of inherited disorders in terms of mode of inheritance (autosomal dominant *vs.* autosomal recessive), age of onset, group of muscles affected and clinical severity. Autosomal recessive TTN mutations are mainly related to early-onset skeletal and/or cardiac muscle disorder.[5] Here we discuss a pediatric patient with infantile hypotonia, dysmorphic findings in the lower extremity, proximal muscle, and facial weakness at whom homozygous frameshift mutation of TTN gene is detected.

Various causative genes can be cited as examples of different types of myopathies. Genes

that may contribute to MmD-type myopathy include RYR1, SELENON, MYH2, MHY7, TTN, CCDC78, UNC45B, ACTN2, and MEGF10.[1,5]

In 1903, published the initial case reports of a congenital muscular dystrophy form. For most congenital muscular dystrophies, the mode of inheritance has been observed as autosomal recessive.[6]

As we discussed earlier, titin the largest protein identified in the human organism, is coded by the TTN gene. Mutations arising within the TTN gene have been intricately linked to a spectrum of musculoskeletal and cardiac pathologies, encompassing hereditary familial dilated cardiomyopathy, autosomal recessive congenital myopathy, and the early onset of respiratory insufficiency, among other debilitating conditions. These genetic alterations in the TTN gene constitute a pivotal nexus in the etiology of these intricate diseases, collectively underscoring the critical role of Titin in both skeletal and cardiac muscle health.[7]

The worldwide incidence of congenital muscular dystrophy remains inadequately documented; nevertheless, Mostacciuolo et al.[8] conducted an assessment in Northeast Italy, suggesting an estimated prevalence of 4.7 cases per 100,000 individuals. Furthermore, a comprehensive investigation into neuromuscular disorders in Northern England, as documented by Norwood et al.[9] in their 2009 publication, projected a general prevalence of congenital muscular dystrophies at approximately 0.89 cases per 100,000 individuals.[10]

Determining the prevalence of limb-girdle muscular dystrophy poses a considerable challenge due to the variability in its features and the potential overlap with symptoms seen in other muscle disorders. Prevalence estimates for this condition span a range from 1 in 14,500 to 1 in 123,000 individuals.[11]

As we've already mentioned and as also known as Salih myopathy is an autosomal recessive hereditary myopathic disorder of the musculature. This condition manifests a constellation of clinical attributes, encompass the early onset of muscle weakness during the neonatal period. Notably, afflicted individuals commonly present with a myopathic facial expression, characterized by an array of phenotypic expressions. This may include variable degrees of ptosis, denoting a drooping of the eyelids. Furthermore, a hallmark feature of Salih myopathy is the delayed attainment of motor developmental milestones, wherein affected individuals exhibit an inability to achieve independent ambulation within the developmental window spanning from 20 months to four years of age. Moreover, this complex clinical profile frequently incorporates spinal anomalies, most notably scoliosis, which may contribute to the musculoskeletal challenges faced by those afflicted. Joint rigidity leading to restricted mobility is another salient manifestation of this condition.

Adding to the clinical complexity of Salih myopathy is its propensity to manifest cardiomyopathic alterations. Particularly noteworthy is the occurrence of dilated cardiomyopathy, a condition characterized by the enlargement of the heart chambers, typically observed in individuals ranging from five to 16 years of age. Beyond this, there exists a considerable risk of significant cardiac arrhythmias, which, if realized, can culminate in untimely mortality prior to the attainment of 20 years of age. This intricate interplay of musculoskeletal and cardiac pathologies underscores the multifaceted nature of Salih myopathy and highlights the importance of comprehensive clinical assessment and management.[12]

TTN gene structure and function

The TTN gene is a colossal gene found in humans and other mammals, comprising 363 exons and 364 intron regions. It is responsible for the expression of the largest protein in humans, known as titin. Additionally, it has been observed that mutations occurring within the TTN gene lead to skeletal muscle and cardiac muscle disorders. This gene holds significance in regulating the contraction of both skeletal and cardiac muscle fibers within the body. This information elucidates why mutations within the TTN gene affect not only skeletal muscles but also cardiac muscles.[13]

The titin protein expressed by the TTN gene serves as a molecular spring in the sarcomeric regions of striated muscles. A sarcomere is defined as the segment between two adjacent

Z bands. In this parallel arrangement, myosin and actin filaments slide past each other, contributing to the regulation of contraction by bringing the two ends of the sarcomere together.[14]

CASE REPORT

A 21-month-old male patient applied to the pediatric neurology clinic with complaints of delayed motor-milestones and difficulty in walking. He was born at 39-week gestational age, from a consanguineous marriage, without any additional prenatal, natal, and postnatal risk factors. He did not experience any respiratory weakness. He had mild swallowing difficulty without malnutrition. He had achieved head-control, independent sitting and standing without support at seven months, 10 months, and 18 months of age respectively. He had recently started walking for 6-7 steps without support.

On physical examination, his vital signs and general medical examination were normal. He had no dysmorphic features. He had myopathic facies and bilateral mild ptosis without ophthalmoplegia. He had axial and appendicular hypotonia. Deep tendon reflexes were absent bilaterally at the lower extremities and diminished bilaterally at the upper extremities. He had both distal and proximal muscle weakness prominent at lower extremities. He had no joint contractures, hyperlaxity, muscle atrophy/hypertrophy, scapular winging or kyphoscoliosis. His ophthalmological examination and brainstem auditory evoked response were normal. At laboratory examination creatine kinase (CK), aspartate aminotransferase (AST), and alanine transaminase levels were normal. In conditions such as muscular dystrophy, it is generally not expected for blood levels of AST, CK, and alanine aminotransferase to remain within normal limits. By examining the results in the context of the patient being a child, an interpretation suggesting that these levels are within the normal range can be made based on the patient's outcomes. Metabolic screening tests (plasma acylcarnitine profile, plasma, and urine amino acid levels, urine organic acid levels, biotinidase activity, vitamin B12, folic acid, homocysteine, plasma lactate, and ammonia) were normal. Electroneuromyography revealed mild myopathic features with normal nerve conduction study. Electrocardiogram and echocardiography were normal. Based on the patient's symptoms in the field of medical genetics, initial suspicion was directed toward spinal muscular atrophy (SMA). Consequently, SMA and multiplex ligation-dependent probe amplification (MLPA) tests were requested, and the results of the examinations were found to be normal. During the anamnesis, it was confirmed that there was no similar case in the child's family. When the child was 19 months old, Sanger sequencing was conducted on the parents. The results of the examination revealed that the child's parents are carriers (heterozygous) for the disease. Following the normal results in terms of SMA, a clinical exome sequencing (CES) test was conducted. Clinical exome sequencing test revealed a novel homozygous NM_001267550.2:c.32676del frameshift variant at 132th exon of the TTN gene. This frameshift variant was considered as potentially pathogenic using in silico pathogenicity prediction tools and has not been found in large reference population databases (ExaC, ESP, 1000G, gnomAD).

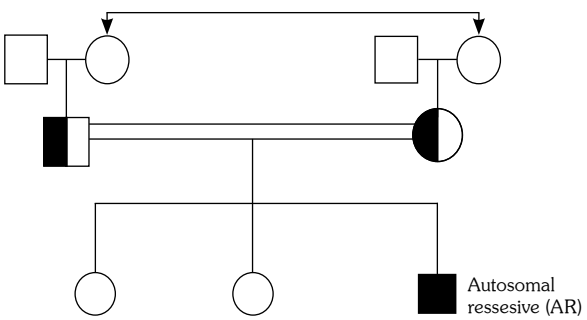

Figure 1. A detailed family pedigree was drawn by getting information from the parents (Created in BioRender.com).

Before the commencement of the study, total deoxyribonucleic acid (DNA) isolation was performed from the samples. Quantification and purity determination of isolated DNA was carried out using spectrophotometric methods during the quality control processes. Additionally, the Qubit fluorometer was employed for quantification purposes.

In the library preparation and sequencing step, the Illumina Nextera DNA Exome kit was utilized. This kit facilitates the sequencing of

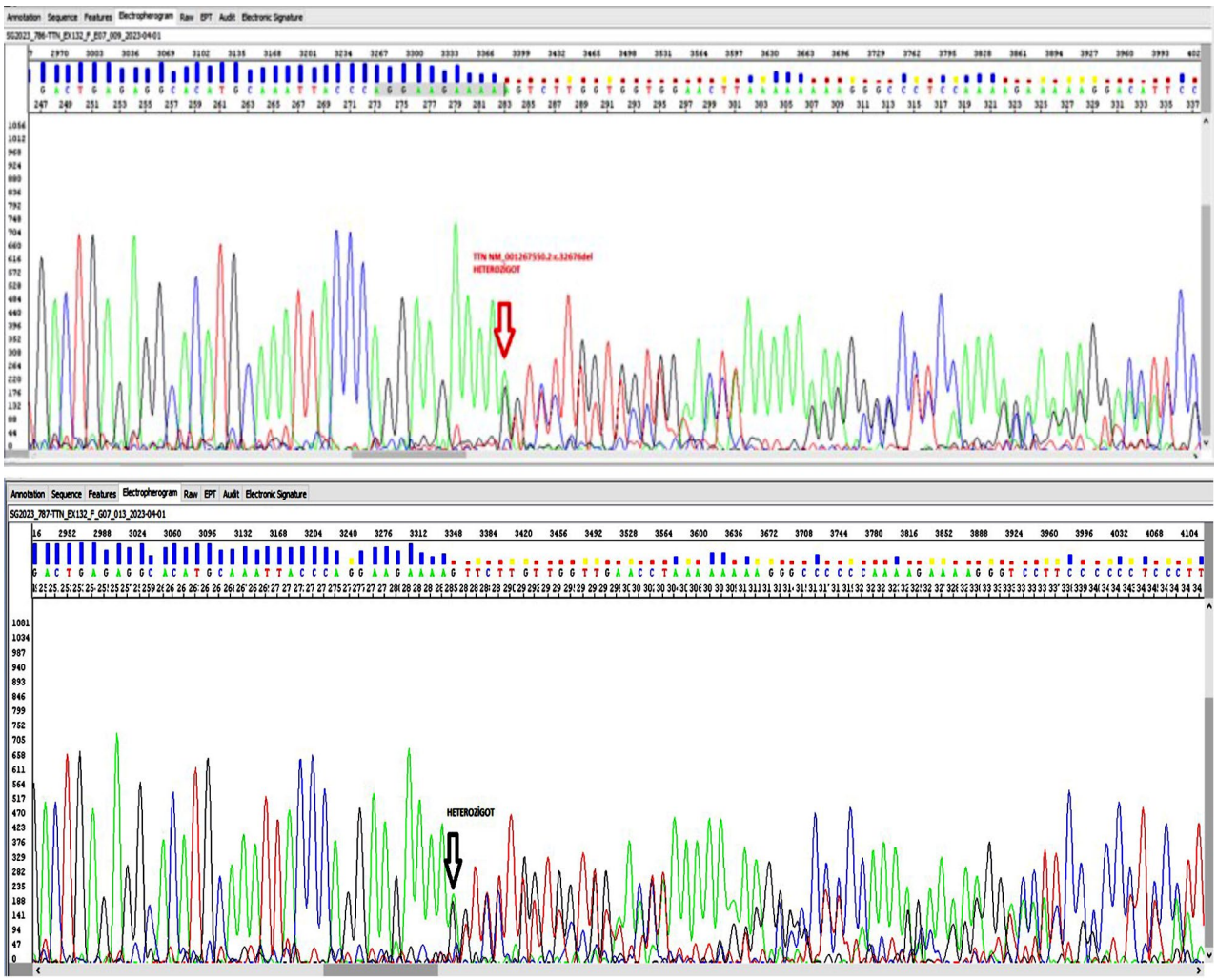

Figure 2. The Sanger sequencing analyses of the patient's parents, revealed the identification of heterozygous variants in both. These variants have been indicated and illustrated in the analysis.

regions on the genome called exomes, which encode proteins. The total size of the target region is 39 megabases. Sequential steps in the library preparation process included fragmentation, addition of index/barcode sequences, amplification, enrichment of exonic regions, and purification. The manufacturer's kit guidelines were followed throughout the study.

For sequencing, the Illumina NextSeq 550 next-generation sequencing platform was employed. In this step, it is planned to obtain +50 million paired-end reads of 2×150 bp per sample. An output of 8 Gb per sample is anticipated, resulting in an average read depth of 100× across the exonic regions. Following sequencing, quality control of the obtained data will be conducted using the FASTQC[15] version 12.0 software (Wellcome Trust Sanger Institute, Hinxton, UK). In order to prevent deviations in subsequent analysis steps due to low-quality base calls in Fastq read data, trimming will be performed.

Trimmomatic application[16] version 0.39 software (GitHub, Inc., California, USA) will be used for quality filtering and trimming processes. Post-trimming alignment will be conducted to the updated human genome sequence

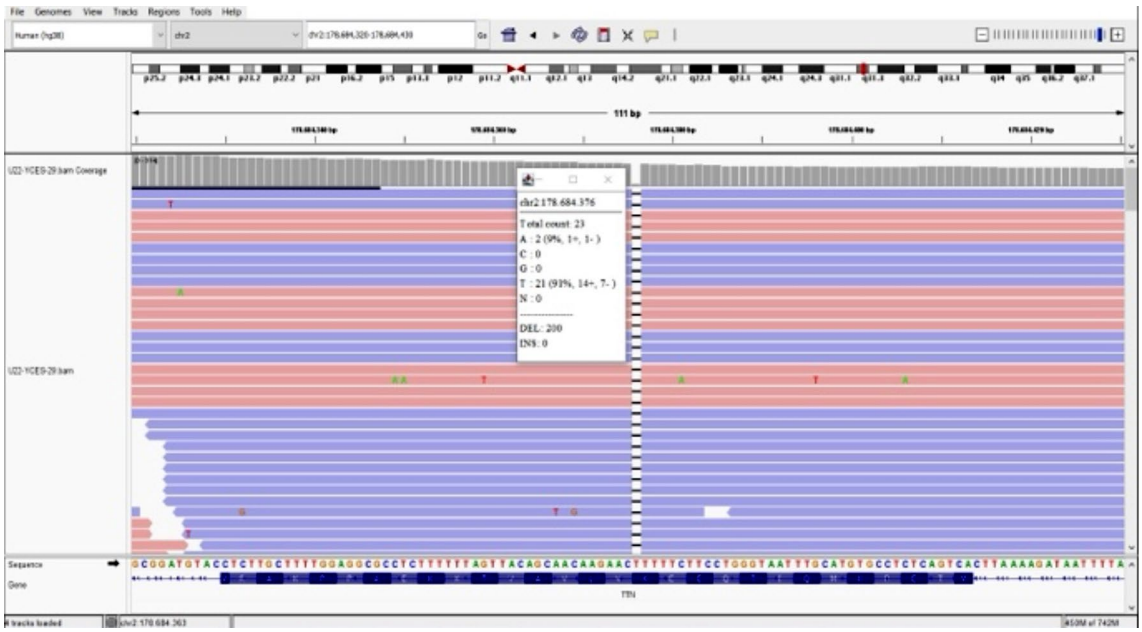

Figure 3. Provides the Integrative Genomics Viewer visualization of the next-generation sequencing (NGS) analysis specific to the proband.

(GRCh38) using the BWA-MEM[15] version 7.13 software (National Institutes of Health, USA). After alignment, filtering procedures based on quality control data will be performed using the Samtools program. Within the analysis, realignment will correct alignment discrepancies in indel regions, recalibration of quality score values for DNA sequences will be executed, optimization of parameters for variations in the organized sequences will be carried out, annotation will be completed for the variation list, and variations with unreliable calls (below 80%) will be filtered out based on strand bias. GATK will be employed for variant calling, and the Variant Effect Predictor program will be used for variant annotation.

Molecular method and analysis

Genomic regions were analyzed using NGS technology. Automated DNA isolation from peripheral blood samples was conducted according to the standard protocols of the QIAAmp DNA Mini (Qiagen, Hilden, Germany) kit. Sequencing was performed on the Illumina NextSeq 500 platform using SOPHIA CES and Illumina V2 chemicals. The next-generation sequencing process, utilizing Illumina SBS (sequence by synthesis) technology, ensures a minimum depth of 50× for each nucleotide. The test evaluates coding exonic regions of relevant genes and the exon-intron boundaries in their vicinity. The method employed may not detect large deletions and duplications. Confirmation of meaningful results through Sanger sequencing and support from functional studies are recommended. Analysis may not provide conclusive results in genomic regions with insufficient total read counts (<50×). Due to the method used, genes may not be fully covered. The ability to detect genetic variants and the interpretation of these variants may vary among laboratories. Laboratory procedures were conducted at high-quality standards to prevent technical and operational errors.

Bioinformatics analyses and variant calling were performed using the Dragen v3.6 bioinformatics analysis program based on the hg19 human reference genome. This analysis evaluated coding exonic regions of the genes included in the study and the exon-intron boundary regions in their vicinity. During the analysis, variants in genes associated with the patient phenotype in the OMIM database and exhibiting an appropriate inheritance pattern were reported from the ClinVar database

(v20200817) as pathogenic, likely pathogenic, or variants of uncertain significance, according to current scientific knowledge. This database is continuously updated, and the data in the report are as of the writing date; changes may occur in the future. Copy number variations were not investigated in this analysis. The interpretation of variants was based on the American College of Medical Genetics and Genomics (ACMG) 2015 guidelines. Due to the lack of sufficient genomic and exomic databases for the Turkish population, the 1000 Genomes Project, dbSNP, and ExAC data were used as control populations. Only genes listed in the ACMG SF v3.1 4 guideline were considered secondary findings from genes outside the indication, and only pathogenic/likely pathogenic variants in genes with an appropriate inheritance pattern were reported. Genetic counseling is recommended before and after testing.

The ability to detect genetic variants and the criteria for interpreting these variants may vary among laboratories. Laboratory studies have been conducted at high-quality standards to prevent technical and operational errors. However, as stated in the informed voluntary consent form, errors such as labeling mistakes, sample-to-sample contamination, inconclusive results, and rare unavoidable errors of human and/or test system origin may occur.

DISCUSSION

Limb-girdle muscular dystrophies are hereditary muscle disorders that affect skeletal and cardiac muscles. As mentioned, the disease can lead to cardiomyopathy due to its impact on the cardiac muscles. Both muscle biopsy and genetic tests can be utilized for diagnosing the disease.[17]

In this case, diagnosis was achieved through genetic testing instead of muscle biopsy. The patient exhibits a homozygous mutation in the TTN NM_001267550.2:c.32676del frame-shift variant. In this condition, while laboratory tests typically show elevated levels of CK, our patient's CK levels were observed within normal limits. We attribute this to the patient's infancy, as they have not initiated motor movements and are exclusively fed with breast milk.

The NM_001267550.2.c.32676del mutation in TTN is listed in ClinVar as potentially pathogenic. Symptom severity associated with this disease can vary in different case studies. During the patient's medical history, it was confirmed that there were no similar cases in the family.[15] The literature suggests affected cases within families of patients with this disease.[18]

A homozygous missense variant in the TTN gene causes autosomal recessive limb-girdle muscular dystrophy type 10. The Sanger analysis of the patient's parents revealed that their asymptomatic condition is explained by their heterozygosity for this autosomal recessive inherited disease. Consanguineous marriages increase the risk of occurrence of such autosomal recessive inherited diseases.[19,20]

In conclusion to other cases, although dysmorphic findings were excluded during the physical examination of our patient, mild ptosis is attributed to the infantile period. The frame-shift mutation in the TTN gene explains the dysmorphic findings in the lower extremities of the infantile patient and rules out suspicion of SMA. As mentioned before, the type of TTN gene mutation seen in congenital limb-girdle dystrophies may vary from patient to patient. The frameshift mutation in the TTN gene detected in our patient explains the patient's dysmorphic findings. As a result of the tests performed on the patient's parents, the fact that the patient is a carrier in terms of the relevant disease supports this situation. When all these were collected, the explanation for the low serum CK level in the tests performed as mentioned before was that our patient had not yet started motor activities. It is worth noting that molecular genetic diagnostic techniques are greatly enhancing disease diagnosis. While muscle biopsy remains a staple in diagnosing various types of muscular dystrophy, genetic analysis tests offer greater convenience in terms of sample collection. Moreover, genetic analyses serve to corroborate findings, proving particularly valuable in investigating disease origins by examining the patient's family history and conducting tests on family members, subsequently facilitating

tailored genetic counseling. In this case report, we utilize molecular genetic tests to diagnose our infant patient and their parents, with the aim of contributing a significant mutation found in our patient to the existing literature.

Patient Consent for Publication: A written informed consent was obtained from the parent of the patient.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions: Idea/concept, other: B.A., S.C.; Design, literature review, writing the article, references and fundings: B.A.; Control/supervision, critical review, materials, data collection and/or processing: S.C.; Analysis and/or interpretation: B.A., S.C., C.B.A.

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