

Novel Gene RPTOR with Pathogenic Mutation Cause: Facioscapulohumeral Muscular Dystrophy–Like Disease

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Abstract**Case Report**

The appearance of next-generation sequencing (NGS) has significantly changed the way we think about scientific approaches in basic research and clinical application. Adult- and adolescent-onset muscular dystrophies (MDs) are a disorders that cause muscle disease (myopathy) characterized by progressive muscle weakness and muscle fibers degeneration (atrophy) which caused by alteration in one or more of genes needed for standard muscle function [5]. Most MDs are congenital disorders, but impulsive alteration can arise. Facioscapulohumeral is a genetic condition caused by abnormal expression of the *DUX4* gene, People with FSHD2 have a mutation in the *SMCHD1* gene. This study presents a new causative novel gene with pathogenic mutation discovered as a de novo mutation, then transferred by Autosomal dominant inheritance to the next generation of first cases. We have identified a heterozygous missense variant of RPTOR (RPTOR:NM_001163034:exon9:c.1093G>A:p.V365I) in this family. After searching we found this mutation to be significant and it is in a gene first time to be reported causing such symptoms and signs of the disease.

Keywords: Facioscapulohumeral muscular dystrophy (FSHD), chromosomal study, RPTOR (novel) is a Coding gene. Whole - exome sequencing (WES).

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INTRODUCTION

Adult- and adolescent-onset muscular dystrophies (MDs) are a group of disorders that cause muscle disease (myopathy) characterized by progressive muscle weakness (myasthenia) and muscle degeneration (atrophy) due to mutations in one or more genes required for normal muscle function [5]. These mutations affect the function of proteins responsible for giving muscle shape and functional support. These conditions can affect other organ systems because some of these proteins are not musculoskeletal-specific. Generally, MDs present with immediate and equal muscle weakness, although others present with distal or regional weakness [1, 2]. They are historically organized by patterns of weakness and inheritance (autosomal dominant, autosomal recessive, X-linked). The classification of muscular dystrophy initially was based on the clinical pattern of weakness, mode of inheritance, and age of onset. With the discovery of genes responsible for most of these diseases, there has been a gradual shift to a classification based on genes affected and function of the respective proteins. The most common adolescent- and adult-onset MDs types

are myotonic MD, Emery-Dreifuss MD, facioscapulohumeral MD, and limb-girdle MDs (subtypes of which include caveolinopathy, dysferlinopathy, and sarcoglycanopathies). Dystrophinopathies (Duchenne's/Becker's MD) typically are discussed as their own subcategory, as are congenital MD such as merosin-deficient MD, alpha-dystroglycanopathies, and Ullrich congenital MD.

Etiology

Most MDs are inherited disorders, but spontaneous mutations can occur. Medical evaluation must include taking a careful history of family members.

Patho-Anatomy/Physiology

The exact relationship between certain mutations and clinical syndromes is being explored. Most of these genes seem to be related to structural integrity of the muscle.

In some cases, these genes are important to tissues other than skeletal muscle. This leads to the

manifestation of clinical features involving the cardiac, pulmonary, gastrointestinal, and central nervous systems [4].

Disease progression, including natural history, disease phases or stages, disease trajectory (clinical features and presentation over time)

Although there are several subtypes of adult-onset MDs, they generally begin with strength loss and decreasing endurance. Patients may develop symptoms at different points in their life. Weakness is mostly in the pelvic and scapular region. Patients may experience falls, difficulty ascending stairs, exercise intolerance, muscle cramps, episodic weakness, focal wasting of muscle groups, contractures, and breathing difficulties [5, 6].

Facioscapulohumeral MD is characterized by muscle weakness and wasting (atrophy). The signs and symptoms usually appear in adolescence. However, the condition's onset and severity varies widely. Milder cases may not become noticeable until later in life, whereas rare severe cases become apparent in infancy or early childhood.

Weakness involving the facial muscles or shoulders is usually the first symptom of this condition. Facial muscle weakness often makes it difficult to drink from a straw, whistle, or turn up the corners of the mouth when smiling. Weakness in muscles around the eyes can prevent the eyes from closing fully when a person is asleep, which can lead to dry eyes and other eye problems. For reasons that are unclear, weakness may be more severe on one side of the face than the other. Weak shoulder muscles tend to make the shoulder blades (scapulae) protrude from the back, a common sign known as scapular winging. Weakness in the muscles of the shoulders and upper arms can make it difficult to raise the arms over the head or throw a ball.

The muscle weakness associated with facioscapulohumeral MD worsens slowly over decades and may spread to other parts of the body. Weakness in the muscles of the lower legs can lead to a condition called foot drop, which affects walking and increases the risk of falls. Muscular weakness in the hips and pelvis can make it difficult to climb stairs or walk long distances. Additionally, weak abdominal muscles may cause an exaggerated curvature of the lower back (lordosis) in affected individuals. Facioscapulohumeral muscular dystrophy (FSHD) detailed pathophysiology is not completely understood. However, it is believed it is caused by inappropriate expression of the double homeobox protein 4 gene (DUX4) [1].

The DUX4 Gene is located in 4q35 region D4Z4 Locus. In normal individuals have 11-150 D4Z4 repeats. But it has been found in cases of FSHD that

only 1-10 D4Z4 macrosatellite repeat arrays are present [1, 2].

In most patients with FSHD one allele of D4Z4 is contracted where the other D4Z4 allele is normal.

FSHD can have different types. For example FSHD1 is caused by the contracted D4Z4 which leads to DNA hypomethylation and a reduction of histone 3 lysine 9 trimethylation and heterochromatin protein 1 gamma markers. This hypomethylation process affects the chromatin structure [3, 4].

In contrast there is FSHD2 which has normal alleles of D4Z4 associated with DNA hypomethylation [3]. Heterozygous mutations in SMCHD1 (structural maintenance of chromosome flexible hinge domain containing 1 gene) are the cause of some if not most cases of FSHD2 via an effect on D4Z4 chromatin structure [5].

CASE PRESENTATION

A 41-year-old female presented to our hospital with progressive muscle weakness gradually increased over years. The patient reported that her symptoms started at age 9 as gradual symmetrical weakness in muscles of facial expression, persisting and progressively worsening over time. This weakness did not affect facial sensation, vision, smell, or ability to swallow. A few years later, the patient started experiencing bilateral proximal weakness on both upper limbs and had difficulty raising her arms and brushing her hair. At 14, the patient went to a hospital for a deltoid muscle biopsy and a full investigation; however, with no conclusive diagnosis, she did not receive any medication and did not follow up. Two years later, the patient became pregnant and began experiencing bilateral proximal weakness in both lower limbs, although mainly on the right side, and started limping and using walking aids. The patient started to experience difficulty in standing from sitting and walking. Soon she was no longer able to walk for long distances and began to use a wheelchair for some time during the day. A few years later the patient did not have the ability to stand and walk again but had no sphincter dysfunction. The patient has had no past medical illness. Although she denies any family history of medical illness, however, her 19-year-old daughter has experienced the same symptoms as her mother, starting at around 7 years old with facial expression weakness and progressing to bilateral proximal upper limb weakness. At 5, her other daughter started complaining of fatigue during the day and of exercise intolerance. On examination, the patient's vitals were stable; heart, lung and abdomen were normal with no neurological sensory deficit; and coordination was normal, with severe wasting of facial, shoulder, and hip muscles. The patient is unable to close her eyes, smile, or close her mouth and has difficulties talking and

eating. With normal tone and reflex, power was 2/5 to 3/5 on proximal muscle and 3/5 to 4/5 on distal muscle.



Fig-1: X-ray of spine shows progressive scoliosis.



Fig-2: MRI spin shows lumbar hyperlordosis

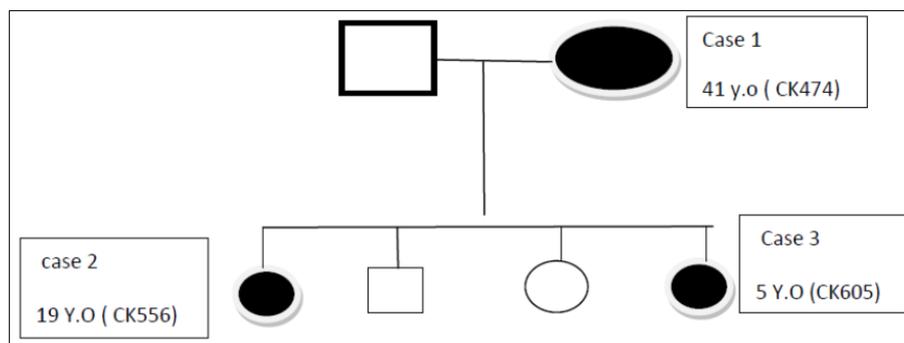


Fig-3: Family pedigree

Molecular

Extensive genetic analysis has been done for this family, initiated by case 2, the older daughter. Her whole exome was sequenced in 2017, and the result was insignificant. The patient repeated the test during a follow-up visit. At the next clinic visit, we proceeded with case 1, the mother, and sent for her whole exome sequencing again at the end of 2017. The genetic analysis revealed significant results. During the next-generation sequencing, the whole exome analysis resulted in identification of a heterozygous missense variant of RPTOR (RPTOR:NM_001163034:exon9:c.1093G>A:p.V365I) in this patient. This variant is thought provoking because of a) the nature of the variant (novel, predicted deleterious in silico) and b) the nature of the gene: pLI score 1.0, RPTOR (regulatory associated protein of MTOR complex 1), the encoded protein forms a stoichiometric complex with the mTOR kinase. The mTORC1 component RPTOR is critical for muscle function and prolonged survival (see PMID: 19046572).

Parental screening to determine the status of the variant as de novo or otherwise is strongly recommended and may support a causative role of this variant in this instance.

The following known Saudi mutation(s) should, according to ACMG guidelines, be classified as pathogenic. This individual presented with incidental

finding of CARRIER status for a known pathogenic mutation. Nucleotide variants (SNVs) with quality scores >350 were reported without Sanger validation. The true positive rate of this class of variant is >99.5%. All novel INDELS were reported only after Sanger validation [6, 7].

RPTOR (regulatory associated protein of MTOR complex 1) is a protein coding gene. Diseases associated with RPTOR include aqueous misdirection and blackwater fever. Among its related pathways are the PI3K-AKT-mTOR signaling pathway and therapeutic opportunities and autophagy pathway. Gene ontology (GO) annotations related to this gene include binding.

Aliases for RPTOR gene

- Regulatory associated protein of MTOR complex 1, 2, 3, 5
- Regulatory-associated protein of MTOR 3, 4
- Raptor 3 4
- P150 target of rapamycin (TOR)-scaffold protein containing WD-repeats 3
- Regulatory associated protein of MTOR complex 1, 2
- P150 target of rapamycin (TOR)-scaffold protein 4

This gene encodes a component of a signaling pathway that regulates cell growth in response to

nutrient and insulin levels. The encoded protein forms a stoichiometric complex with the mTOR kinase and also associates with eukaryotic initiation factor 4E-binding protein-1 and ribosomal protein S6 kinase. The protein positively regulates the downstream effector ribosomal protein S6 kinase and negatively regulates the mTOR kinase. Multiple transcript variants encoding different isoforms have been found for this gene [provided by RefSeq, Sep 2009].

The RPTOR gene is involved in the control of the mammalian target of rapamycin complex 1 (mTORC1) activity, which regulates cell growth and survival and autophagy in response to nutrient and hormonal signals; it functions as a scaffold for recruiting mTORC1 substrates. mTORC1 is activated in response to growth factors or amino acids. Growth-factor-stimulated mTORC1 activation involves an AKT1-mediated phosphorylation of TSC1-TSC2, which leads to the activation of the RHEB GTPase that potently activates the protein kinase activity of mTORC1. Amino acid-signaling to mTORC1 requires

its relocalization to the lysosomes mediated by the regulator complex and the regulation of GTPases.

Genomic locations for RPTOR gene

chr17:80,544,819-80,966,373 (GRCh38/hg38)

Size: 421,555 bases

Orientation: Plus strand

chr17:78,518,619-78,940,173(GRCh37/hg19)

Size: 421,555 bases

Orientation: Plus strand

Genomic view for RPTOR gene

Genes around RPTOR on UCSC Golden Path with Gene Cards custom track

Cytogenetic band:

17q25.3 by HGNC

17q25.3 by Entrez Gene

17q25.3 by Ensembl

RPTOR Gene in genomic location: bands according to Ensembl, locations according to GeneLoc (and/or Entrez Gene and/or Ensembl if different).

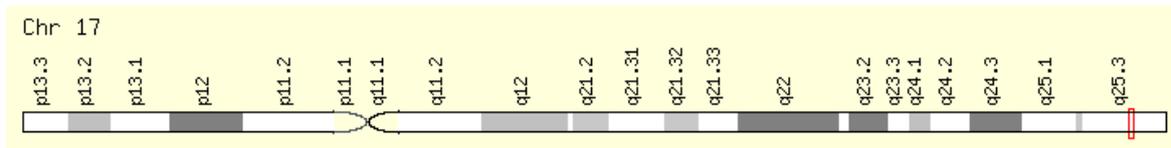


Fig-4: Locations for RPTOR gene

Protein attributes for RPTOR gene

Size: 1335 amino acids

Molecular mass: 149038 Da

Quaternary structure

Part of the mammalian target of rapamycin complex 1 (mTORC1), which contains MTOR, MLST8, RPTOR, AKT1S1/PRAS40, and mTORC1, binds to and is inhibited by FKBP12-rapamycin. Binds directly to 4EBP1 and RPS6KB1 independently of its association with MTOR. It also binds preferentially to poorly or non-phosphorylated forms of EIF4EBP1, and this binding is critical to MTOR's ability to catalyze phosphorylation. mTORC1 forms a complex with

MTOR under both leucine-rich and -poor conditions. It interacts with ULK1 in a nutrient-dependent manner; the interaction is reduced during starvation. It also interacts (when phosphorylated by AMPK) with 14-3-3 protein, leading to inhibition of its. It interacts with SPAG5; SPAG5 competes with MTOR for RPTOR binding, resulting in decreased mTORC1 formation. Finally, mTORC1 interacts with WAC; WAC positively regulates MTOR activity by promoting the assembly of the TTT complex composed of TELO2, TTI1, and TTI2 and the RUVBL complex composed of RUVBL1 and RUVBL2 into the TTT-RUVBL complex, leading to the dimerization of the mTORC1 complex and its subsequent activation.

Expression for RPTOR gene

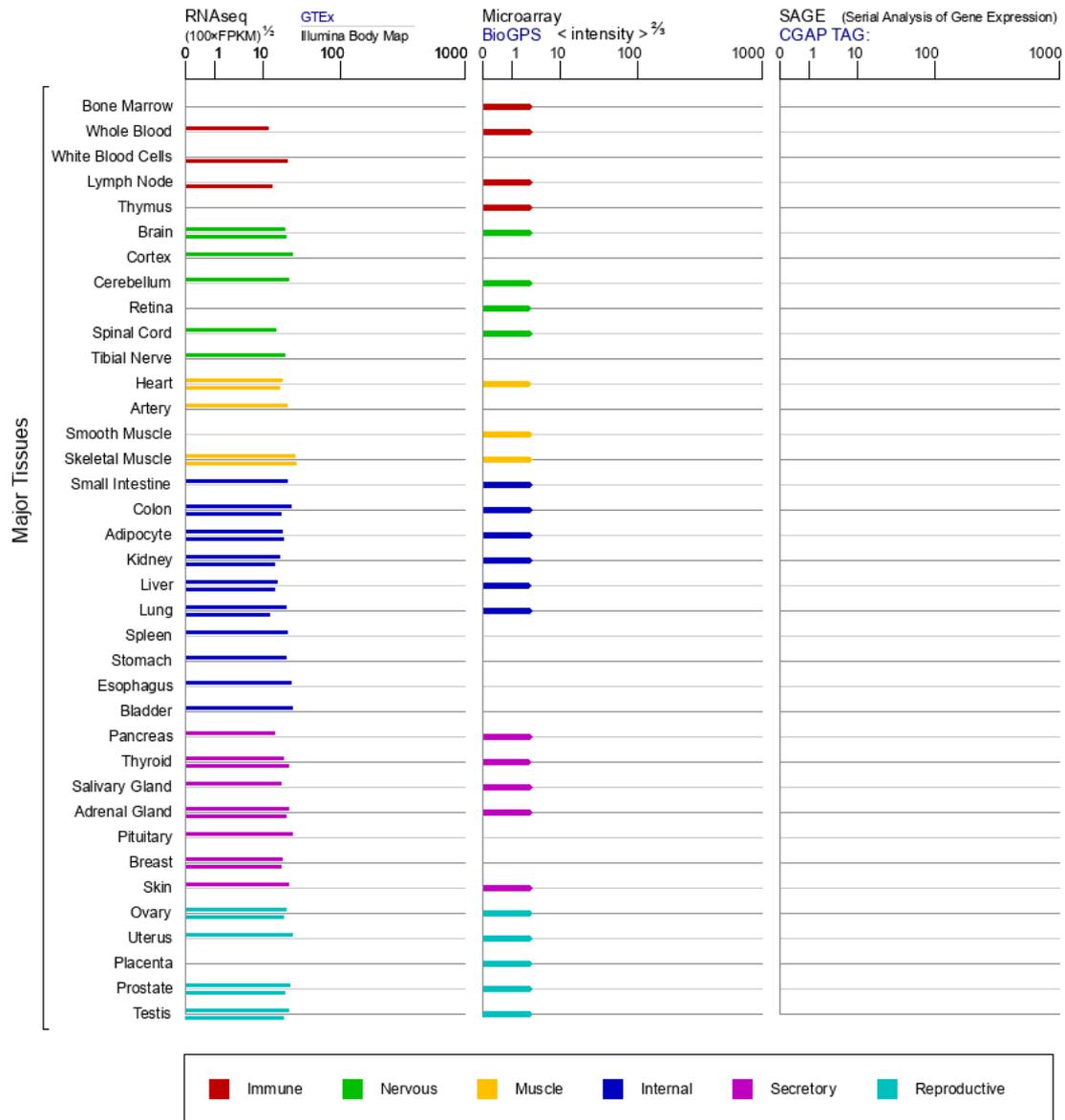


Figure-5

CONCLUSION

The application of next-generation sequencing as a research and diagnostic strategy has made significant progress in solving many problems. This study presents a new causative novel gene with pathogenic mutation discovered as de novo mutation that is transferred by x-linked inheritance to the next generation.

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Disclosures: None.

Footnotes

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