THE EUROPEAN SOCIETY OF MEDICINE
Medical Research Archives, Volume 13 Issue 6

RESEARCH ARTICLE

Digenic inheritance of variants in *RYR1* and *PLEC* causing myopathy with tubular aggregates

Leema Reddy Peddareddygari[1], Arman Singh Grewal[2], Raji P. Grewal[1,3*]

[1]Dynamic Biologics Inc., 1 Deer Park drive, Monmouth Junction, New Jersey, USA, 08852
[2]Larner College of Medicine, University of Vermont, Vermont, USA, 05401
[3]Capital Institutes for Neurosciences, 100K. Johnson Blvd North, Suite 201, Bordentown, New Jersey, USA, 08505

OPEN ACCESS

PUBLISHED
30 June 2025

CITATION
Peddareddygari, L R., et al., 2025. Digenic inheritance of variants in *RYR1* and *PLEC* causing myopathy with tubular aggregates. Medical Research Archives, [online] 13(6). https://doi.org/10.18103/mra.v13i6.6564

DOI
https://doi.org/10.18103/mra.v13i6.6564

ISSN
2375-1924

ABSTRACT

Despite the advances in genetic testing, a significant proportion of patients with suspected genetic myopathies remain undiagnosed. In these patients, the standard patterns of transmission of single gene disorders which include autosomal dominant, autosomal recessive or X-linked are investigated. However, an under recognized phenomenon that could be considered is digenic inheritance in which variants in two separate genes established to cause myopathy result in the phenotype. We report a 53-year-old man who had complaints of fatigability and weakness. Following neurological examination and electrophysiological testing he was diagnosed with a myopathy. Muscle biopsy confirmed the presence of a myopathy with tubular aggregates without inflammatory features, and a genetic etiology was suspected. There was no family history to suggest an autosomal dominant pattern of inheritance and no family members available for further study. Whole exome sequencing was performed and revealed variants in multiple genes including the *ryanodine receptor 1* (*RYR1*) gene, *Kelch repeat and BTB (POZ) domain containing 13*, (*KBTBD13*) gene, *spectrum repeat containing nuclear envelope protein 1* (*SYNE1*) gene and *plectin* (*PLEC*) gene. These variants were further analyzed for available published research papers and databases reporting pathogenicity, and frequency. In addition, we performed modeling for possible pathogenicity and protein/protein interactions for each of the variants. We hypothesize that in this patient, the ryanodine receptor 1 (*RYR1*) gene variant, c.1598 G>A, and plectin (*PLEC*) gene variant, c.2144 C>T following digenic inheritance are the cause of his genetic myopathy. Our study demonstrates that genetic diagnosis can be facilitated by recognition of digenic inheritance in patients with undiagnosed myopathies and the utility of the tools to analyze variants even if family members are not available for study.

Keywords: Digenic inheritance, myopathy, tubular aggregates, *RYR1* gene and *PLEC* gene

Introduction

Classically genetic myopathies have been classified according to the mode of inheritance, X-linked, autosomal recessive or autosomal dominant. However, the revolution in genetic sequencing and testing has allowed classification and diagnosis based upon the affected gene. Despite improvements in DNA sequencing technology resulting in high throughput genetic analysis, a significant portion of patients with possible genetic myopathy remain uncharacterized[1,2,3]. It has been suggested that digenic inheritance could account for some of the patients suspected of having a genetic basis for undiagnosed neurological disorders including myopathies[4,5].

Digenic inheritance is a genetic mechanism in which variants in two different genes result in a disorder. This is the simplest model of inheritance involving multiple genes in which one variant alone is not sufficient to cause a given phenotype but requires the presence of an additional variant in a different gene[6]. There are number of potential pathophysiological mechanisms that include indirect interactions through a shared biochemical pathway, direct interactions in the encoded proteins or epistatic interactions where one gene modifies the effect of the other[7]. With the advances in whole exome sequencing, gene panels are commercially available in which multiple genes known to cause a given phenotype can be simultaneously sequenced and analyzed. For example, in a genetic myopathy panel, more than 70 genes established to cause myopathies can be available for study. This facilitates investigation of digenic inheritance as specific genes with variants can be prioritized for further analysis based upon reported phenotypes and protein interactions [4,8,9].

In this study, we report a patient with an undiagnosed myopathy characterized predominantly by fatigability and discomfort that we hypothesize follows a pattern of digenic inheritance.

Case Study

This is a 53-year-old man who was seen in neurological consultation for complaints of discomfort involving his arms and legs. The patient was referred by an infectious disease physician who had taken care of this patient for more than twenty years for management of HIV disease. In addition to the complaints of discomfort in his muscles, the patient had a history of frequent falls due to weakness and instability in his legs. There was a significant component of fatigability in his complaints. He did not have any complaints of difficulty chewing, swallowing, speaking or with double vision. He denied having numbness or tingling in his arms or legs. He has never seen a neurologist before for any reason with no relevant history of stroke, seizure or neuropathy.

His infectious disease physician could not relate his complaints to any aspect of his disease or with the medications used to treat the disease. All the parameters monitoring his HIV were not significant. He had been on anti-HIV medications which were held, and this made no difference to his symptoms. He was also taking a statin medication for hyperlipidemia and when it was held, there was no change in his complaints.

Neurological examination revealed good use of speech and language and a normal mental status. The cranial nerve examination testing cranial nerves 2-12 revealed no abnormalities. The reflex examination was +2 at the biceps, triceps, brachioradialis, patellae with 1+ ankle jerks and flexor plantar responses. Sensory examination was normal to light touch pinprick vibration and proprioception at the great toes. Cerebellar function as assessed by finger-nose-finger testing and heel shin testing was normal. Power testing showed good strength except for equivocal weakness (Medical Research Council scale 5-/5) of internal rotation and external rotation in his arms and hip flexion in both legs. In addition, the patient could not stand or walk on his heels or toes.

There was no family history to suggest a myopathic or neurogenic process.

Routine blood work including a cell count and differential was normal. A series of CPK levels were performed and showed mild elevation ranging from 500-800 U/L (normal-24-204). An electromyogram (EMG) performed at an outside institution and was reported as normal. This was repeated at our institution. In our study, sensory potential testing the right median, ulnar, radial, superficial peroneal and bilateral sural nerves were normal. Motor nerve parameters in the right median, ulnar and peroneal nerves were also normal. In the right tibial nerve, recording the abductor hallucis muscles, there were normal distal latencies, reduction of the response amplitude (2.3 mV; normal >4.0 mV) and normal conduction velocities. A needle EMG of selected muscles of the right arm and both legs was performed. No abnormal spontaneous activity was observed in any muscles sampled. The presence of low amplitude polyphasic units and a full interference pattern with maximal effort was observed in the shoulder girdle muscles tested including the deltoid, biceps and the supraspinatus and infraspinatus muscles. In addition, similar findings were noted in the vastus lateralis muscles as well as tibialis anterior and medial gastrocnemius muscles. Overall, the study was interpreted as showing evidence suggestive of a mild noninflammatory myopathy.

A muscle biopsy was performed of the left vastus lateralis muscle and showed no evidence of a mitochondrial DNA depletion myopathy which can be a complication of anti-HIV therapy. There was no evidence indicative of myositis, vasculitis or other inflammatory features, however the presence of tubular aggregates was noted in approximately 10 to 15% of fibers.

Given the patient's symptoms, neurological examination, blood test results, EMG testing and muscle biopsy there is strong evidence suggesting that the patient had genetic myopathy rather than an acquired inflammatory myopathy.

Genetic testing and Analysis

The patient underwent genetic testing through a commercial company testing more than 70 genes known to cause myopathy. Variants were found in multiple genes including the *ryanodine receptor 1* (*RYR1*) gene, c.1598 G>A, *Kelch repeat and BTB (POZ) domain containing 13*, (*KBTBD13*) gene, c.382 T>C, *spectrum repeat containing nuclear envelope protein 1* (*SYNE1*) gene, c.241 C>T and *plectin* (*PLEC*) gene, c.2144 C>T. Unfortunately, the patient's family members were not available or interested in further testing to determine which of these variants were inherited from which parent. This kind of issue arises frequently and can be addressed by close examination of each of the variants and correlating it to the phenotype observed supplemented by additional data such as, in this case, the muscle biopsy results.

We further analyzed these variants for frequency and the potential effects of the changes on protein structure or/and function employing protein modelling tools, SIFT[10], Polyphen-2[11] and mutation taster[12]. Furthermore, using STRING analysis[13] we determined potential interactions between the proteins encoded by these genes.

The *RYR1* variant c.1598 G>A (p.Arg533His), rs144336148, results in a non-conservative amino acid change. It has a variant allele frequency A=0.000107 (27/251492) in GnomAD exome database, there is an entry in ClinVar as variant of uncertain significance (Variation ID: 133103). Protein modelling tools predict the variant to be tolerated by Sift, probably damaging by PolyPhen-2. In contrast, mutation taster predicts this variant to be disease causing based upon a splice site change. There are known disease mutations at this position (HGMD CM992212). For example, this *RYR1* variant c.1598 G>A has been reported in the literature in at least three individuals affected with malignant hyperthermia[14,15]. These reports do not provide data about an association of this variant with myopathy. A different substitution at the same amino acid (p.Arg533Cys) is reported to segregate

with in-vitro contracture test results in a large three generation family[16]. Furthermore, it has been reported that this variant results in increased sensitivity to ryanodine receptor agonist[17]. In general, it has been noted that exon 15 is a hotspot in the *RYR1* gene for pathogenic malignant hyperthermia variants. Our analysis provides strong evidence that this mutation is pathogenic and contributing to the disease phenotype in our patient. However, there is no family history to suggest that carrying this mutation alone can result in his phenotype. We hypothesize that there is a contribution to his phenotype from one of the other variants detected in his genotype.

The *KBTBD13* gene variant rs572875121, c.382 T>C, p.Phe128Leu, has a low frequency, C=0.000121 (17/140016) GnomAD database. This polymorphism has been reported in ClinVar (variation ID: 1426480) as a variant of uncertain significance. Analyzing the variant with protein modelling tools predicts this variant to be deleterious by SIFT, however PolyPhen-2 and mutation taster do not support its pathogenicity. We assigned a low priority in terms of contribution to the phenotype in our patient.

The *SYNE1* gene variant, rs375917264, c.241 C>T, p. Arg81Cys, has a frequency of A=0.000080 (20/250808) in the GnomAD exome database. In the ClinVar database (Variation ID: 285295) it is reported as a variant of uncertain significance. Protein modelling using SIFT predicts this variant as deleterious, PolyPhen-2 as possibly damaging and mutation taster as pathogenic. This variant has not been reported in the literature in individuals affected by *SYNE1*-related conditions.

The *PLEC* gene variant found in this patient, rs377358791, c.2144 C>T, p.Pro715Leu (ENST00000436759.2), has an allele frequency of A=0.000201 (48/238948) in the GnomAD exome database. The variant is also reported in the ClinVar database (Variation ID: 496999, transcript ENST00000354589.3, p.Pro688Leu) as a variant of uncertain significance. Protein modelling indicates the variant as deleterious by SIFT, PolyPhen-2 predicted it as unknown, while mutation taster tool predicts it to be a disease-causing variant. Similar to the *SYNE1* gene variant, this variant has also not been reported in the literature in individuals affected with *PLEC*-related conditions.

Discussion

Our analysis shows that based on frequency and effects of the variants as assessed by protein modeling, both the *SYNE1* and *PLEC* genes are good candidates as potential contributors to the disease phenotype. To provide further insight into the potential role of these two variants we subjected them to further analysis using the STRING analysis tool. This tool allows an exploration of possible interactions between the *SYNE1*, *RYR1* and *PLEC* proteins. There are potential interactions between all these proteins that function in muscle physiology. The RyR protein is located in the junctional sarcoplasmic reticulum and releases Ca^{+2} ions into the cytosol leading to muscle contraction. Both *SYNE1* and *PLEC* are structural proteins that participate in muscle contraction and mutations in both can result in muscular dystrophy. Mutations in *SYNE1* can result in Emery-Dreifuss muscular dystrophy which is often characterized by muscle contractures, cardiac defects and progressive weakness[18]. *PLEC* related muscle disorders exhibit a relatively unusual feature of prominent fatigability. This is a significant prominent symptom in this patient suggesting that this gene may be involved in his phenotype[19]. Based upon clinical features, we give higher priority to this gene compared with *SYNE1* in the disease pathophysiology in our patient.

Tubular aggregate myopathy refers to a morphological feature found on muscle biopsies and has been described in a variety of conditions including both autoimmune and genetic disorders. A genetic basis occurs in about 1/3 of all cases and pathophysiology is thought to involve calcium homeostasis[20]. Although no case of tubular aggregate myopathy due to a *RYR1* mutation has been described, the role of *RYR1* in calcium metabolism makes it a plausible candidate contributing to the development of these aggregates. In addition, in

an investigation of plectin in muscle fibers with cytoarchitectural abnormalities, low binding for plectin in tubular aggregates was reported[21]. A number of morphological abnormalities have been reported in patients with muscle related plectinopathies[19] including one patient with sarcolemmal nuclear aggregates. This suggests that dysfunctional plectin protein could contribute to the pathophysiology of tubular aggregates as observed in the muscle biopsy of our patient.

Conclusion

In this patient, we propose the combination of the variants in *RYR1* and *PLEC* have resulted not only in his phenotype of weakness and fatigability but also the presence of tubular aggregates on the muscle biopsy. Our study shows the importance of considering digenic inheritance in patients with suspected genetic myopathies of unclear etiology. An analysis can be performed without additional family members who may not always be available. Given the advances in sequencing technologies, genomics and proteomics, it has been suggested that there now may be a role for machine learning improving the ability to establish digenic inheritance and to make a specific diagnosis[22]. This is important, as an exact diagnosis allows for appropriate genetic counseling and management of patients.

Conflicts of Interest Statement:

The authors have no conflicts of interest to declare.

Funding Statement:

No financial disclosure.

Acknowledgments:

No acknowledgements

References:

1. Nallamilli BRR, Chakravorty S, Kesari A, et al. Genetic landscape and novel disease mechanisms from a large LGMD cohort of 4656 patients. *Ann Clin Transl Neurol.* 2018;5(12):1574-1587. doi: 10.1002/acn3.649.

2. Chakravorty S, Nallamilli BRR, Khadilkar SV, et al. Clinical and Genomic Evaluation of 207 Genetic Myopathies in the Indian Subcontinent. *Front Neurol.* 2020; 11:559327. doi: 10.3389/fneur.2020.559327.

3. Töpf A, Johnson K, Bates A, et al. Sequential targeted exome sequencing of 1001 patients affected by unexplained limb-girdle weakness. *Genet Med.* 2020;22(9):1478-1488. doi: 10.1038/s41436-020-0840-3.

4. Peddareddygari LR, Grewal RP. Digenic inheritance in patients with undiagnosed myopathies. *Medical Research Archives,* 12(11) 2024. doi:10.18103/mra.v12i11.6046.

5. Posey JE, Harel T, Liu P, et al. Resolution of Disease Phenotypes Resulting from Multilocus Genomic Variation. *N Engl J Med.* 2017;376(1):21-31. doi: 10.1056/NEJMoa1516767.

6. Schäffer AA. Digenic inheritance in medical genetics. *J Med Genet.* 2013;50(10):641-52. doi: 10.1136/jmedgenet-2013-101713.

7. Neuhofer CM, Prokisch H. Digenic Inheritance in Rare Disorders and Mitochondrial Disease-Crossing the Frontier to a More Comprehensive Understanding of Etiology. *Int J Mol Sci.* 2024;25(9):4602. doi: 10.3390/ijms25094602.

8. Deltas C. Digenic inheritance and genetic modifiers. *Clin Genet.* 2018;93(3):429-438. doi: 10.1111/cge.13150.

9. Kerner G, Bouaziz M, Cobat A, et al. A genome-wide case-only test for the detection of digenic inheritance in human exomes. *Proc Natl Acad Sci U S A.* 2020;117(32):19367-19375. doi: 10.1073/pnas.1920650117.

10. Ng PC, Henikoff S. Predicting deleterious amino acid substitutions. *Genome Res.* 2001;11(5):863-74. doi: 10.1101/gr.176601.

11. Adzhubei IA, Schmidt S, Peshkin L, et al. A method and server for predicting damaging missense mutations. *Nat Methods.* 2010;7(4):248-9. doi: 10.1038/nmeth0410-248.

12. Schwarz JM, Cooper DN, Schuelke M, Seelow D. MutationTaster2: mutation prediction for the deep-sequencing age. *Nat Methods.* 2014;11(4):361-2. doi: 10.1038/nmeth.2890.

13. Szklarczyk D, Kirsch R, Koutrouli M, et al. The STRING database in 2023: protein-protein association networks and functional enrichment analyses for any sequenced genome of interest. *Nucleic Acids Res.* 2023;51(D1): D638-D646. doi: 10.1093/nar/gkac1000.

14. Ibarra M CA, Wu S, Murayama K, et al. Malignant hyperthermia in Japan: mutation screening of the entire ryanodine receptor type 1 gene coding region by direct sequencing. *Anesthesiology.* 2006;104(6):1146-54. doi: 10.1097/00000542-200606000-00008.

15. Miller DM, Daly C, Aboelsaod EM, et al. Genetic epidemiology of malignant hyperthermia in the UK. *Br J Anaesth.* 2018;121(4):944-952. doi: 10.1016/j.bja.2018.06.028.

16. Tammaro A, Bracco A, Cozzolino S, et al. Scanning for mutations of the ryanodine receptor (RYR1) gene by denaturing HPLC: detection of three novel malignant hyperthermia alleles. *Clin Chem.* 2003;49(5):761-8. doi: 10.1373/49.5.761.

17. Sato K, Roesl C, Pollock N, Stowell KM. Skeletal muscle ryanodine receptor mutations associated with malignant hyperthermia showed enhanced intensity and sensitivity to triggering drugs when expressed in human embryonic kidney cells. *Anesthesiology.* 2013;119(1):111-8. doi: 10.1097/ALN.0b013e31828cebfe.

18. Zhang J, Felder A, Liu Y, et al. Nesprin 1 is critical for nuclear positioning and anchorage. *Hum Mol Genet.* 2010;19(2):329-41. doi: 10.1093/hmg/ddp499.

19. Zrelski MM, Kustermann M, Winter L. Muscle-Related Plectinopathies. *Cells.* 2021;10(9):2480. doi: 10.3390/cells10092480.

20. Fan Q, Gwathmey K, Du X, Seth A, Corse A. Tubular aggregate myopathy causing progressive fatiguable weakness. *Pract Neurol*. 2024;24(2):137-140. doi: 10.1136/pn-2023-003838.

21. Vita G, Monici MC, Owaribe K, Messina C. Expression of plectin in muscle fibers with cytoarchitectural abnormalities. *Neuromuscul Disord*. 2003;13(6):485-92. doi: 10.1016/s0960-8966(03)00037-3.

22. Okazaki A, Ott J. Machine learning approaches to explore digenic inheritance. *Trends Genet*. 2022;38(10):1013-1018. doi: 10.1016/j.tig.2022.04.009.