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CASE REPORT

Digenic inheritance in patients with undiagnosed myopathies

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ABSTRACT

Advances in genetic sequencing have allowed a specific gene-based diagnosis in many patients with a suspected genetic myopathy. However, a significant minority of patients remain in whom the specific diagnosis remains elusive. In addition to the classic forms of Mendelian inheritance including autosomal recessive, autosomal dominant and X-linked, another mechanism of genetic transmission is digenic inheritance. This occurs when there is an interaction of two genes resulting in a given phenotype. In this study, we report three patients with myopathy hypothesized to follow a digenic pattern of inheritance. All of the patients had progressive weakness due to a myopathic disorder and underwent genetic analysis of a panel of genes established to cause inherited myopathies. Variants were observed in multiple genes in this panel. The data was further analyzed by review of published reports of the variants, public databases to determine the frequency and subjected to protein modeling to assess their possible pathogenic significance. One patient had variants in the *CAPN3* (c.1553A>G; pGln518Arg) and *SYNE2* (c.7664C>T; p.Thr2555Met) genes that our analysis indicates are the likely cause of her myopathy. Following a similar protocol, a second patient carries genes with two variants that are in cis in the *TTN* gene (c.85582G>A; p.Val28528Ile and c.103292C>T; p.Thr34431Met). There is another variant in the *SYNE2* gene (c.18632C>T;p.Thr6211Met) and the combination of both likely result in her myopathy. In the last patient, a previously described mutation in the *COL6A2* gene, c.1970-3 C>A in intron 25 was identified. This can result in limb girdle muscular dystrophy phenotype observed in this patient. In addition, a new variant was detected in the *SGCG* gene, c.643 G>T, p.Ala215Ser and its role as a modifier of disease phenotype is discussed. Our study suggests that digenic inheritance should be considered in patients with suspected undiagnosed myopathies in whom next generation sequencing fails to establish a diagnosis.

Keywords: Digenic inheritance, genetic myopathy, myopathy genes, *CAPN3, SYNE2, TTN, COL6A2, SGCG.*

Introduction

Classically genetic myopathies were classified according to the mode of inheritance, autosomal recessive, autosomal dominant and X-linked[1]. However, with the revolution in genetic sequencing and testing the newer classification schemes are based upon the affected gene[2]. These improvements in genetic sequencing technologies are discussed with a historical perspective in a study of Dutch families diagnosed with limb girdle muscular dystrophy and followed for twenty-one years[3]. The role of whole exome and targeted sequencing in facilitating and increasing the ability to make a specific genetic diagnosis has been reported[4-6]. However, despite these improvements which do result in high throughput genetic data, a significant portion of patients with possible genetic myopathy remain uncharacterized[7-9].

An underrecognized phenomenon that may be a factor in these undiagnosed patients is digenic inheritance[10]. In broad terms, digenic inheritance refers to the genetic mechanism by which mutations in two different genes are required for phenotypic expression. In this study, we report three patients with an undiagnosed myopathy who we hypothesize developed following a pattern of digenic inheritance.

Case studies

PATIENT 1

This patient was referred for a neuromuscular second opinion regarding progressive weakness. She was a 79-year-old woman who was in good health until 5 years earlier when at age 74 years, she developed back pain and underwent a laminectomy for spinal stenosis. She recovered from the surgery but was left with mild numbness in her feet. However, following this procedure she was living independently. During this time, also at age 74 years, she developed an anaphylactic reaction to amoxicillin prescribed for dental procedure resulting in loss of consciousness and admission to a hospital. A neurological examination was requested due to a change in mental status. By the time she was seen, her neurological examination showed that her mental status was normal. Power testing showed intact strength in the upper extremities and distally in her legs. Minimal weakness of hip flexion was noted but not graded and this was present prior to her lumbosacral spine surgery. Sensory testing was intact for cold and vibration at the ankles. A cranial CT scan showed no acute pathology.

Over the next few years, she neurologically declined and had increasing difficulty in ambulating. After further evaluation elsewhere, she was diagnosed with a possible demyelinating neuropathy and treated with IVIG for 2 months. She did not respond to treatment and continued to decline.

Her general medical history was significant for hypertension, pulmonary embolus and atrial fibrillation. She was placed on Eliquis. She continued to progress and was referred to a university neuromuscular center. An evaluation was done at that institution and an electromyography (EMG) was performed. The nerve conduction study showed evidence suggestive of a length-dependent sensorimotor neuropathy. A repetitive stimulation study was negative demonstrating no evidence of a disorder of the neuromuscular junction. However, a needle examination was performed, and the patient was diagnosed with a "non-irritative myopathy" and a muscle biopsy was recommended. The patient continued to decline and became wheelchair dependent. One year later, a muscle biopsy was performed of the quadriceps and showed severe myofiber atrophy with a background of increased interstitial adipose tissue which infiltrated into the muscle bundles. Scattered fibers also exhibited altered fibrillar/intra fibrillar organization and overall, the findings were consistent with a longstanding myopathy.

When she presented for a neurological examination to our center, she was 79 years old. She could not stand without assistance or dress herself. She denied any complaints of difficulty chewing swallowing or speaking. She did complain of weakness in her arms and legs and of numbness in her feet.

Neurological examination showed a normal mental status with no evidence of ptosis and normal extraocular eye movements. She could not stand and had marked weakness grade 0/5 MRC scale testing hip flexion, hip abduction, abduction knee flexion and extension, 3/5 testing foot dorsiflexion eversion and inversion. In her arms she had weakness of finger extension with relative preservation of the finger flexors 4/5 weakness of the biceps, deltoid, internal rotation and external rotation. She was areflexic with down going toes. She had a decrease in vibration and proprioception in a stocking distribution worse distally in the feet. No tremors were noted, and she had no contractures.

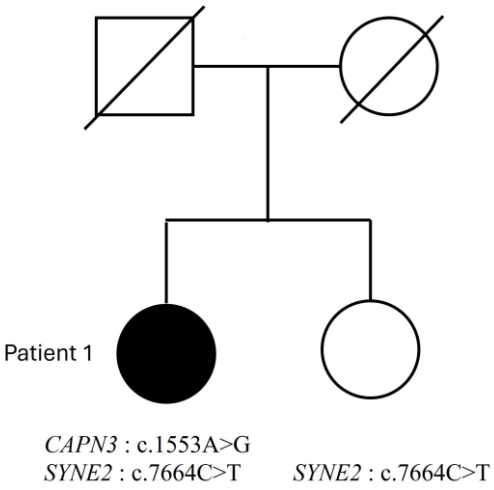
Family history was negative for any neuromuscular disorder suggestive of neuropathy or myopathy. Given her rate of neurological decline, a tapering dose of steroids was prescribed starting at 60 mg a day and tapered over 2 months. This did not result in any improvement of her power.

Throughout her multiple neurological evaluations, a number of blood tests were done and were negative or normal: CBC and differential, comprehensive metabolic panel, levels of vitamin B12, folate, thiamine, vitamin B6, hemoglobin A1c, serum protein electrophoresis and immunofixation and serology for hepatitis C, HIV and Lyme disease. In addition, given her prior diagnosis of neuropathy and lack of response to IVIG, a diagnosis of CMT had been proposed. Genetic testing performed through a commercial company analyzing more than twenty-five genes was negative showing no mutations or variants in any of the genes analyzed.

A repeat EMG nerve conduction study was done at our institution and unequivocally showing the presence of a myopathy without inflammatory features. We hypothesized that she may have a genetic myopathy.

Commercial genetic testing was ordered to investigate the genetic etiology of her muscle disease. This panel consisted of more than thirty-five genes established to cause myopathy and detected variant in the *Calpain-3* (*CAPN3*), rs764593698, c.1553A>G (p. Gln518Arg) and rs201297144, c.7664C>T (p. Thr2555Met) variant in *Spectrin Repeat Containing Nuclear Envelope Protein 2* (*SYNE2*) gene. A second variant was not found in either of these two genes. The patient's sister was available for neurological examination and at age 76 years had no evidence of neuropathy or myopathy. Following IRB approved policies and procedures, she underwent genetic testing for the *CAPN3* and *SYNE2* variants. She was discovered to carry only the *SYNE2* (c.7664C>T; p. Thr2555Met) variant (Figure 1)

Figure1. Pedigree of Patient 1. Squares indicate males, circle indicate female, Dark fill indicate affected individual, Line across indicate deceased individual. Patient 1 has inherited both *CAPN3* and *SYNE2* variants while her unaffected sister has inherited only the *SYNE2* variant.



Further analysis showed that the *CAPN3* variant has a low frequency reported at G=0.000008 (1/121258) in gnomAD global population study group[11]. The amino acid glutamine at 518 position in CAPN3 protein is highly conserved across species. Variants were further analyzed using protein modelling tools, SIFT[12], PolyPhen-2[13] and mutation taster[14]. Analyzing the p.Gln518Arg variant, predicted this variant as tolerated by SIFT, however it was predicted to be possibly damaging by PolyPhen-2 and disease causing by mutation taster analysis. The *SYNE2* variant has been observed in T=0.000133 (15/113028) in gnomAD data[11], all of them were heterozygous. SIFT predicts that this variant would not be tolerated, PolyPhen-2 analysis indicated this variant as possible damaging while mutation taster indicates that this variant is likely a polymorphism. STRING is a database of known and predicted protein-protein interactions[15]. It shows that the proteins encoded by *CAPN3* and *SYNE2* genes have a common molecular function with cytoskeleton protein binding and mutations in both these genes are associated with muscular dystrophy.

PATIENT 2

This patient was a 50-year-old woman who was referred for a neuromuscular second opinion with a complicated past medical history. Two years earlier, at age 48 years, she was having difficulty with weakness in her arms and legs and shortness of breath. She was admitted to a local hospital and no etiology was found for either her shortness of breath or weakness. Although she was able to climb up and down stairs, she felt weak. One year later she worsened and was re-admitted to hospital. She was evaluated by both a cardiologist and a pulmonary physician and no etiology for weakness or shortness of breath was found. She was transferred to a rehabilitation facility with ongoing difficulties with shortness of breath and the inability to climb up stairs. She had been having some complaints about swallowing problems over the prior six months. Her neurological history was unremarkable, and she had no history of a stroke, seizures or more recent complaints of double vision. Her general medical history was significant for hypertension and heartburn. The patient has two children who are healthy and parents who were both aged 83 years and had no neurological problems. There is no extended member of the family that had any neurological problems.

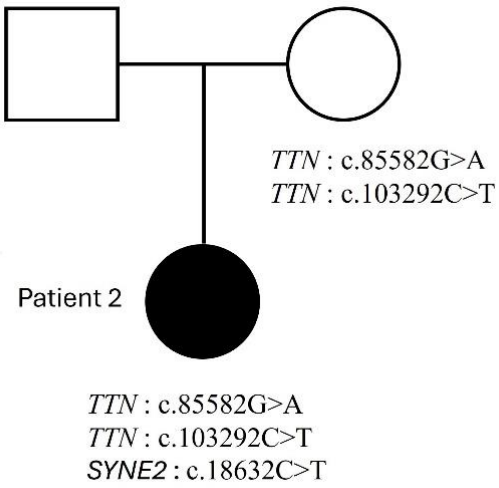
When the patient was examined at our facility, she was noted to be morbidly obese at 290 pounds and a height of 5 feet 3 inches. Neurological examination revealed a normal mental status and cranial nerve examination. She was diffusely hyporeflexic with flexor plantar responses. Sensory examination was normal to light touch, pinprick, vibration and proprioception tested at the great toes bilaterally. Motor examination revealed good strength in her arms testing both proximal and distal musculature but weakness MRC grade 4/5 of the proximal leg muscles bilaterally. The patient was not able to stand on her toes or heels and had difficulty walking more than ten yards due to shortness of breath and weakness. She also started to use a walker.

Routine blood work including a creatine phosphokinase and aldolase were within normal limits. An EMG was done of both arms and the nerve conductions was normal. However, the needle EMG disclosed evidence of a myopathic process without significant inflammatory features. A muscle biopsy was recommended but the patient declined. Based on the EMG, a potential diagnosis of genetic myopathy was considered. She underwent commercial genetic testing for thirty-seven genes known to cause myopathy and a number of variants were discovered. These included two in the *Titin* (*TTN*) gene (rs753861250, c.85582G>A; p. Val28528Ile and rs192001910, c.103292C>T; p.Thr34431Met), one each in the *SYNE2* gene (rs36215895, c.18632C>T; p.Thr6211Met) and the *structural maintenance of chromosomes flexible hinge domain containing protein 1* (*SMCHD1*) gene (c.2505T>C) which represents a synonymous change with no amino acid change. All the variants result in an amino acid change except for the *SMCHD*1 gene variant. The

TTN and *SYNE2* variants were prioritized and the *SMCHD1* variant was considered low priority in terms of contributing to the pathophysiology of the myopathy. Further analysis of the *TTN* variants indicates (c.85582G>A; p. Val28528Ile has a frequency of 0.000002 (4/1613188) in gnomAD data[11]. Protein modelling with SIFT[12], PolyPhen-2[13] and mutation taster[14] indicate that this is a pathogenic variant. The *TTN* (c.103292C>T; p.Thr34431Met) variant has been reported with a frequency of 0.0009159 (1478/1613770) in gnomAD data[5]. Protein analysis tools, SIFT, PolyPhen-2 and mutation taster predict the variant as damaging variant. The *SYNE2* variant has a frequency of T=0.00493 (329/66794, ALFA) (https://www.ncbi.nlm.nih.gov/snp/rs36215895). Protein analysis tools, SIFT, PolyPhen-2 and mutation taster tools indicate that this is a possibly damaging variant. STRING analysis[15] shows that the proteins encoded by *TTN* and *SYNE2* genes have a common molecular function with cytoskeleton protein binding and mutations in these genes are associated with muscular dystrophy.

The patient's mother was neurologically normal and agreed to participate in the study. Following IRB approved policies and procedures, she underwent commercial genotyping. This revealed that she carried both *TTN* variants indicating the two *TTN* variants are in cis (Figure 2). She did not carry the *SYNE2* variant suggesting the patient inherited this allele from her father.

Figure 2. Pedigree of patient 2. Squares indicate males, circle indicate female, Dark fill indicate affected individual. The unaffected mother has both the *TTN* variants, the affected daughter, patient 2, has inherited both the *TTN* variants from the mother and inherited *SYNE2* variant from the father.



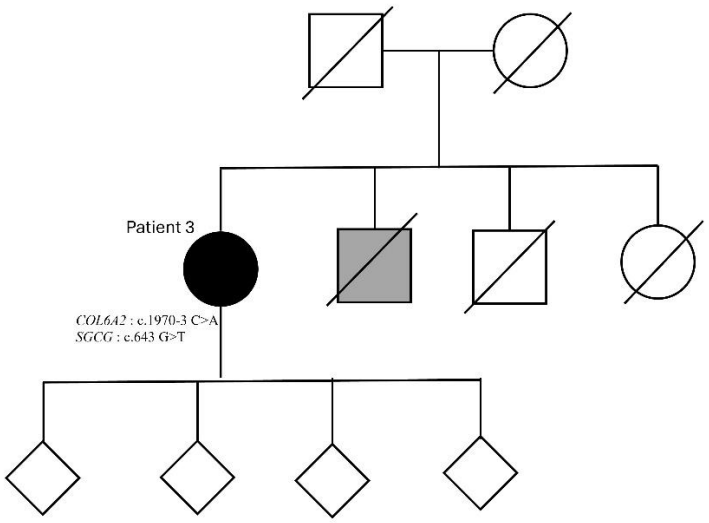
PATIENT 3

This patient presented at age 57 years with episodes of falling. She had a long history of diabetes which was diagnosed at age 23 years and subsequently had heart disease ultimately having bypass surgery at age 48 years. She also underwent gastric bypass surgery and lost more than 60 pounds at age 55 years. She was admitted to hospital and a neurological examination revealed significant sensory loss to all modalities in a stocking distribution worse distally. She had proximal weakness in her legs which was 5 on MRC scale testing hip adduction/abduction, 4/5 hip flexion, 5 hip extension, knee flexion and extension and 4+ distally testing foot dorsiflexion/plantar flexion. She had gait imbalance, could not walk on her heels or toes and could not perform a tandem walk. She had a positive Romberg. She had an EMG performed at an outside facility which revealed sensorimotor polyneuropathy. Ultimately, she was referred because her endocrinologist felt that her neurological disability was not in keeping with her history of diabetes and relatively good diabetic control.

She was examined at age 60 years and continued to have complaints of numbness tingling, weakness and imbalance. Her neurological examination was similar to that documented three years earlier however, at present she could not walk without assistance. She used a walker and a motorized scooter for mobility. Review of prior records and additional tests ordered were negative or normal for a comprehensive neuropathy workup including tests for vitamin levels, serum protein electrophoresis, autoimmune antibodies such as antiganglioside antibodies and serology for HIV. A repeat EMG was performed and showed evidence of a sensorimotor polyneuropathy and a length-dependent pattern worse in the lower extremities but without ongoing active denervation. Needle EMG disclosed chronic neurogenic changes including the proximal and distal muscles of the legs and the distal muscles of the arms. Sampling the proximal muscles of the arms revealed mild evidence of a neurogenic process with large motor units observed. Overall the study was interpreted as showing evidence of chronic sensorimotor polyneuropathy with a combination of demyelinating and axonal features. However, a co-existing myopathic process could not be excluded.

The patient had a family history significant for neurological disorders without clear diagnosis. Her parents died from heart disease with no history of any neurological disorder. She had two brothers and one sister who were all deceased; one brother had a progressive neurological disorder and died in his twenties. He had been given the diagnosis of muscular dystrophy, but this was unsubstantiated. She has four children with a variety of diagnoses including autism and seizures but no history to suggest a peripheral neurological disorder. No family member was available for examination or testing (Figure 3).

Figure 3. Pedigree of Patient 1. Squares indicate males, circle indicate female, diamond indicates gender of individual is unknown. Dark fill indicates affected individual, grey fill indicates possibly affected, Line across indicate deceased individual. Patient 3 has two variants, one each in *COL6A2* and *SGCG* genes.



Given the unclear family history with her brother possibly suffering from a peripheral neurological disorder, commercial genetic testing was ordered for both a hereditary neuropathy and a muscular dystrophy panel. A hereditary neuropathy panel which consists of sixty-four genes known to cause neuropathy were tested and a single variant was detected and reported as a variant of unknown significance in the *Enhancer of polycomb-like protein 1* (*EPL1*) gene, c.360 C>G p.D120E but no other variant found in this gene or any of the other sixty-three genes tested.

Another panel testing ninety-nine genes known to cause myopathy showed one variant each in the *collagen type VI alpha 2 chain* (*COL6A2*) gene, rs201879417 c.1970-3 C>A and the *sarcoglycan gamma* (*SGCG*) gene c.643 G>T, p. Ala215Ser.

The *COL6A2* variant, rs201879417 has been previously described with an allele frequency of A=0.000977 (112/114628) in gnomAD data[11]. This variant is in intron 25 located 3bp from the splice site and predicted as disease causing by mutation taster analysis[14]. The *SGCG* gene variant c.643 G>T, p. Ala215Ser is a new variant and reported in 1/105318 allele frequency in gnomAD data[5]. Protein modeling predicted this variant as deleterious by SIFT[12] and PolyPhen-2[13] analysis predicted it to be benign and mutation taster analysis predicted this as a disease-causing variant. STRING analysis[15] shows that the proteins encoded by *COL6A2* and *SCGC* genes are components of sarcolemma and mutations in these genes are associated with limb-girdle muscular dystrophy.

Discussion

Recent studies have varying degrees of success with high throughput sequencing in diagnosing patients with suspect genetic myopathies. Westra et al[16] studied 396 individuals with neuromuscular symptoms and confirmed a diagnosis of genetic myopathy in 19% of them representing 75 patients. Topf et al[9] report on 1001 patients, of which a specific genetic diagnosis was achieved in 488 patients. Interestingly, they did report that 18 patients in this cohort had variants in two genes, however, they did not discuss the possible relevance of this finding. In contrast, there are several studies in which the significance of this finding is discussed. In 2018, Nalliamilli et al.[7] reported on 4,656 patients recruited from the United States with clinically suspected Limb-girdle muscular dystrophy and analyzed with next-generation sequencing. In this group, a genetic diagnosis was achieved in 27% of patients. However, it was noted that in this cohort, thirty-one patients had pathogenic variants in two separate genes. In a more recent report published in 2020, with the study population based in the Indian subcontinent, 207 patients with a suspected genetic myopathy were studied[8]. In this population a specific diagnosis was achieved in 49%. In thirteen patients, pathogenic variants were carried in two separate genes. In both publications, the issue of a possible digenic inheritance is discussed as possibility leading to a specific diagnosis. However, these were studies of individual patients with no analysis of family members making it difficult to investigate possible digenic inheritance.

Digenic inheritance refers to the genetic mechanism by which a patient will develop a given disease when mutation on separate genes is co-inherited. Inherent in this process is the concept that a single mutation is not enough to result in the disorder. The phenotype of the disorder is a result of two mutations in separate genes that occur simultaneously. We have previously described the approach that we used in a patient with limb girdle muscular dystrophy due to digenic inheritance[17].

Both Patients 1 and 2 have a history, neurological examination and investigations that indicate a progressive myopathy. In Patient 1, the presence of both *CAPN3* (c.1553A>G; pGln518Arg) and *SYNE2* (c.7664C>T; p. Thr2555Met) are noted in her but not in the unaffected sister who only carries the *SYNE2* (c.7664C>T; p.Thr2555Met) variant. After our analysis of variant frequency, protein modeling and STRING analysis, we suggest that she suffers from a myopathy following digenic inheritance. In Patient 2, there are two variants in the *TTN* gene (c.85582G>A; p. Val28528Ile and c.103292C>T; p.Thr34431Met), one in the *SYNE2* gene (c.18632C>T;p.Thr6211Met). The *TTN* gene is one of the largest in the human genome, with 31636 germline variants of which 553 are reported as pathogenic variants, 3207 as "Likely pathogenic" and 11258 variants of "Uncertain significance" (https://www.ncbi.nlm.nih.gov/clinvar/?term=TTN+gene accessed Sep 2024). Our analysis shows that they are both potentially disease producing but since her mother, who is neurologically normal carries both *TTN* variants, suggesting they are not enough to result in a genetic myopathy. However, our patient inherited *SYNE2* gene (c.18632C>T; p. Thr6211Met) variant from her neurologically normal father. As in Patient 1, our analysis suggests that her myopathy is likely due to a combined effect of *TTN* and *SYNE2* variants.

Patient 3 represents a challenge because she does have a neuropathy and therefore additional testing such as muscle biopsy would likely not be diagnostically helpful. This is because her study would likely show neurogenic changes as indicated by her EMG test. However, as noted by her endocrinologist, the severity of her diabetes did not correlate with her neurological deficits and it is likely that she does have an associated myopathy causing proximal muscle weakness in her legs. Interestingly, her genetic testing revealed a mutation in the *COL6A2* gene, c.1970-3 C>A in intron 25. In addition, a new variant was detected in the *SGCG* gene, c.643 G>T, p. Ala215Ser reported as a variant of unknown significance. A different variant, rs770020910, c.643 G>A, p. Ala215Thr has been previously reported in SGCG gene at the same location in 2/105318 alleles in gnomAD data[11].

The *COL6A2* gene encodes one of several chains which constitute type VI collagen. This protein forms part of the extracellular matrix and is important for maintaining skeletal muscle function and integrity. Mutations in this gene have been reported to cause myopathy following both an autosomal dominant or recessive pattern and a broad range of phenotypes which can be relatively mild such as Bethlem myopathy or severe including Ullrich congenital muscular dystrophy. This c.1970-3 C>A mutation has been reported in two patients with Bethlem myopathy[18,19] and in five heterozygous patients with limb-girdle muscular dystrophy type 1A. Overall, given the lack of contractures and other features, this patient follows the pattern of limb-girdle muscular dystrophy type 1A rather than Bethlem myopathy.

Our study of these patients suggests digenic inheritance as a possible mechanism for patients with previously undiagnosed myopathies. As indicated by the study of patients 1 and 2, to investigate such genetic mechanism, family members must be available and willing to participate in a study. Chakravorty et al[8] had noted variants in multiple genes known to cause a genetic myopathy and even proposed digenic inheritance contributing to the phenotype in some of their study participants. However, given the nature of their investigation, family members were not included in the study population limiting their ability to follow up to perform further analysis to confirm the possibility of digenic inheritance.

Conclusion

We suggest that digenic inheritance should be considered in patients with suspected genetic myopathies. In such patients in whom multiple variants are detected in genes known to cause inherited muscle disorders, further analysis could implicate some of these variants as contributing to disease pathophysiology. The results of such analysis could provide a genetic diagnosis which in turn has implications for genetic counseling of such patients.

Conflict of Interest Statement:

The authors have no conflicts of interest to declare.

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