An atypical presentation of ACAD9 deficiency: Diagnosis by whole exome sequencing broadens the phenotypic spectrum and alters treatment approach

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Acyl-CoA dehydrogenase 9 (ACAD9), linked to chromosome 3q21.3, is one of a family of multimeric mitochondrial flavoenzymes that catalyze the degradation of fatty acyl-CoA from the carnitine shuttle via β-oxidation (He et al. 2007). ACAD9, specifically, is implicated in the processing of palmitoyl-CoA and long-chain unsaturated substrates, but unlike other acyl-CoA dehydrogenases (ACADs), it has a significant role in mitochondrial complex I activity. The diagnosis of ACAD9 deficiency was initially considered, due both to these findings and to her atypical presentation. Biochemical assay for ACAD9 deficiency is not clinically available. Family trio-based whole exome sequencing (WES) identified 2 compound heterozygous mutations in the ACAD9 gene. This discovery led to optimized treatment of her mitochondrial dysfunction, and supplementation with riboflavin, resulting in clinical improvement.

There have been fewer than 25 reported cases of ACAD9 deficiency in the literature to date. We review these and compare them to the unique features of our patient. ACAD9 deficiency should be considered in the differential diagnosis of patients with lactic acidosis, seizures, and other symptoms of mitochondrial disease, including those with normal mitochondrial enzyme activities. This case demonstrates the utility of WES, in conjunction with biochemical testing, for the appropriate diagnosis and treatment of disorders of energy metabolism.

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1. Introduction

The β-oxidation of fatty acids is catalyzed by the activity of acyl-CoA dehydrogenases, a family of multimeric flavoenzymes, located in the mitochondria. All of the ACADs are nuclear-encoded proteins that are transported to the mitochondria where they assume a mature function in branched chain amino acid catabolism and fatty acid oxidation [17]. Each ACAD enzyme is specific for a select fatty acyl-CoA, requiring a particular carbon chain length and saturation status. ACAD9 is a recently discovered enzyme that preferentially processes unsaturated fatty acyl-CoAs with 14–20 carbons [32]. The breakdown product of β-oxidation is primarily acetyl-CoA which can be funneled into the TCA cycle to produce ATP via the electron transport chain, or ketone bodies for use by the brain and heart during times of fasting. The importance of ACAD9 in fat oxidation has recently come into question, with studies showing that knockdown of ACAD9, alone, does not lead to detectable changes in acylcarnitine profiles [25].

ACAD9, unlike the other ACADs, is implicated in the assembly of mitochondrial complex I, the first step in the electron transport chain and oxidative phosphorylation [26]. Mitochondrial complex I is an essential component of cellular energy production via electron transfer from...
metabolic acidosis with elevated lactate and increased anion gap that
occurred with no intracranial hemorrhage, and blood work revealed a severe
acidosis. Upon admission, CT scan revealed a right parietal skull frac-
ture [9,12,15,20]. For disorders of energy metabolism such as ACAD9 de-
ciency with multiple phenotypes, variable severity, and age of
onset, whole exome sequencing has been an essential tool in guiding
the patient’s diagnosis and treatment [15].

2. Clinical synopsis

Our patient is a globally developmentally delayed female child who
presented at 11 months of age with a 4-day history of dystonic postur-
ing as well as a 1-day history of altered level of consciousness. She had
stiffening of the upper and lower extremities, without ocular or oral in-
volvement. Upon admission, CT scan revealed a right parietal skull frac-
ture with no intracranial hemorrhage, and blood work revealed a severe
metabolic acidosis with elevated lactate and increased anion gap that
resolved with fluid resuscitation.

She was the 7-pound 8-ounce product of an uncomplicated 40-week
gestation, born to a 30-year-old G3P2 mother, with both parents of
Guatemalan origin and without consanguinity. There was no family his-
tory of mitochondrial or genetic disorders. Both of the patient’s older
brothers were healthy.

Initial laboratory and imaging results revealed normal plasma quan-
titative amino acids, newborn screens, EEG, and brain MRI, however
urine organic acids showed an elevation in 3-hydroxybutyrate.

At the follow-up visit, her dystonic posturing continued and her de-
velopment had not progressed. MECP2 DNA sequencing was normal;
however, serum lactate was elevated at 5.4 mmol/L (normal 0.5 to
2.2). Serum pyruvic acid levels were normal at 1.16 mg/dL (normal 0.30 to
1.50). EEG was without irregularities.

Over the next 8 months, the patient’s symptoms and development were
relatively unchanged, with shaking spells of the extremities, last-
ing 2 min, that occurred when the patient was given sweets. Neurolog-
ic examination showed poor truncal tone, normal passive tone in the
arms, and spasticity in her legs. There was titubation of her head and a
tremor in her hands. Tendon reflexes were hyperactive, with a persist-
ten “striatal toe” sign on the right, and Babinski’s sign on the left.

LP with CSF collection for metabolic and neurotransmitter studies
indicated alterations in many amino acids, including CSF alanine at
73.9umol/L (normal 12.6–34.7), and an increased CSF lactate of
5.9 mmol/L (normal 0.8–2.4). Skin fibroblast studies indicated normal
Pyruvate Dehydrogenase Complex activity at 2.66 nmol/min/mg (nor-
mal 1.26–4.42). Muscle biopsy revealed increased lipid and oxidative
enzymes. EM showed increased number, size, and abnormal
morphology of mitochondria, consistent with mitochondrial disease.
Mitochondrial myopathy profile showed NADH dehydrogenase was
26.55 mcg/ml/min/mg (normal 5.78–23.70), NADH Cytochrome c Reduc-
tase was 1.80 mcg/ml/min/mg (normal 0.41–1.21), Succinate Dehydro-
genase was 1.67 mcg/ml/min/mg (normal 0.45–1.29), Succinate Cytochrome
c Reductase was 2.66 mcg/ml/min/g (normal 0.42–1.65), Cytochrome c
Oxidase was 7.84 mcg/ml/min/g (normal 1.03–3.83), and Citrate Syn-
thase was 38.01 mcg/ml/min (normal 6.86–24.62). Her serum lactate was
7.4 mmol/L (normal 0.5–2.2) and serum pyruvate was 1.46 mg/dL
(normal 0.30–1.50) on a free-flowing vein. Her plasma amino acids re-
vealed an alanine of 959umol/L (normal 119–523). On MRI, bilateral T2
hyperintensities in the cerebral peduncles and small bilateral peaks on
MR spectroscopy at the posterior parietal white matter were observed,
which may be seen in metabolic syndromes such as Leigh syndrome
(Fig. 1). Furthermore, ophthalmologic exam revealed cortical visual im-
pairment as well as optic nerve atrophy, raising concerns for a mito-
ochondrial condition. Treatment with levocarnitine, Co-enzyme Q10,
and vitamin E were started, and the patient’s fatigue improved, and day-
time napping decreased. However, her other symptoms, including neck
arching and trunk hypotonia, did not change.

The child and parents were recruited into a research protocol at the
Translational Genomics Research Institute (TGen). As described in detail
below, whole exome sequencing was performed on this family trio, re-
vealing compound heterozygosity for the V59F and I166W variants in
the ACAD9 gene.

Based on the whole exome sequencing results, in conjunction with our
patient’s clinical, biochemical, radiological, and histopathological
findings, even in the absence of mitochondrial complex I deficiency, we
determined that ACAD9 deficiency was the correct diagnosis for our
patient.

Because ACAD9 deficiency typically presents with cardiac dysfunc-
tion due to hypertrophic cardiomyopathy, an echocardiogram was per-
formed. It revealed normal cardiac anatomy with mild left ventricular
hypertrophy, left ventricular mass greater than 95th percentile, and
brisk systolic function. The patient remained asymptomatic from a car-
diac standpoint.

Her parents continued to note tremors, staring spells, frequent falls,
and difficulty walking with no change in her dystonia or truncal hypoto-
nia. She had persistent failure to thrive for which high calorie nutritional
supplements, and medium chain fatty acid oils were started and a G-
tube placed.

Levetiracetam was started to treat seizures. Her seizure control im-
poved with optimization of medication dosage. She now displayed
choreaathetoid-like movements of the upper extremities and neck.
High dose riboflavin (200 mg/d) was also initiated for improvement of
mitochondrial complex I function which is typically diminished in
ACAD9 deficiency [13].

With physical therapy, she was able to progress from bearing weight in
the standing position, to ambulating with a walker. At her most re-
cent visit, our patient had some improvement in her neck and truncal
hypotonia. She was able to stand and walk with support. Her weight
was in the 1st percentile and height in the 15th percentile.

3. Materials and methods

3.1. Ethics

Patient and parents were enrolled into a clinical research protocol
sponsored by the Translational Genomics Research Institute (TGen)
aproved by the Western Institutional Review Board, Protocol Number
20120789. After obtaining informed consent, blood was collected for
DNA and RNA extraction. An aliquot of the proband’s DNA sample was
sent for Sanger sequencing and independent confirmation of selected
variants in a clinical laboratory (GeneDx, Gaithersberg, MD).

3.2. Whole exome sequencing

DNA was extracted from the blood of both parents and proband. Exome libraries were prepared with the Illumina TruSeq Exome
Enrichment kit v2, following the manufacturer’s protocol. Sequencing
was performed by 101 bp paired-end sequencing on a HiSeq2000
instrument (Illumina Inc., San Diego, USA). Filtered reads were aligned to the Human genome (Hg19/GRC37) using the Burrows-Wheeler transform (BWA-MEM). Reads where sorted and PCR duplicates were removed using Picard, and base quality recalibration, indel realignment were performed using the Genome Analysis Toolkit (GATK). Variants were called jointly with UnifiedGenotyper, annotated with dbnsFP and snpEff for protein-coding events. Prediction scores were loaded from dbNSFP and used for filtering.

An annotated variant file was created that included variants in any of the three family members (female child, father, and mother). The list was filtered to include novel, private or rare variants according to the Exome Aggregation Consortium (ExAC) database (ExAC Browser v.0.3 [January 2015 release]). Variants predicted to be benign by Combined Annotation Dependent Depletion (CADD) tool from University of Washington and Polyphen2 were removed.

3.3. Protein modeling

Protein modeling was done using the technique outlined by Nouws et al. 2010 (also found at http://www.cmbi.ru.nl/~hvensela/ACAD9/) [6, 26]. Jmol modeling software was used to visualize the predicted ACAD9 model [5].

3.4. Article selection

Articles in Table 1 were selected based on reported cases on the ClinVar database (http://www.ncbi.nlm.nih.gov/clinvar) under the search term “ACAD9”. Only mutations associated with a publication which included patient phenotype were included in the table. The articles not found on this database were found via a search of the PubMed database with the term “ACAD9”. Only cases with descriptions including at least 4 of the 6 categories of patient or ACAD9 deficiency related information (gender, age, mutation, symptoms, age at onset, blood lactate), including symptoms, were included in the table. Blood lactate was the only laboratory finding present in the table due to the presence of that parameter in almost every single case. Other parameters, such as pyruvate, ammonia, AST, ALT, and others were not consistently reported. The lactate levels displayed were those reported on either initial examination or, if not reported on initial presentation, those with the maximum value reported.

4. Results

The proband had no de novo variants. There were 6 heterozygous variants consistent with functional mutations, not resulting in a disease phenotype: FAM231B, LNP1, C4orf6, ADAM29 (a metalloproteinase-disintegrins), KAT6B, and DCHS1. KAT6B is an autosomal dominant disorder associated with Genitopatellar syndrome (GTPTS), a rare disorder consisting of microcephaly, severe psychomotor retardation, and characteristic coarse facial features, and urogenital anomalies. Our patient’s KAT6B variant allele, was inherited from her father, and is not known to cause Genitopatellar syndrome. DCHS1 encodes a transmembrane cell adhesion molecule that belongs to the protocadherin superfamily.

Variants in four genes were consistent with a compound heterozygous model: HLA-A, NCOA3 (nuclear receptor activator), PLK5, and ACAD9. Susceptibility to Stevens-Johnson syndrome, allopurinol-induced severe cutaneous adverse reaction, and carbamazepine-induced hypersensitivity syndrome have been associated with HLA-
is predicted

V59F variant is predicted to be among the top 0.1% most deleterious substitutions in the human genome. The CADD scores were 23 for the V59F variant and 26.4 for the L166W variant, which places these mutations in the category of being both novel and private. The CADD scores were 23 for the V59F variant and 26.4 for the L166W variant, which places these mutations in the category of being both novel and private. Neither variant is found in the ExAC Browser database, which contains information on 121,779 non-Asian individuals.

Mitochondrial exome was also sequenced and no clinically significant variants were identified.

The patient was a compound heterozygote for variants V59F and L166W in the ACAD9 gene. The V59F (c.175 G > A) variant was inherited from her mother, and the L166W (c.497 T > G) variant was inherited from her father and the L166W variant.

ACAD9 gene, localized on chromosome 3q21.3, consists of 18 exons [1,4]. The encoded ACAD9 protein is a 621 amino acid dimer with an altering α-helical architecture, unlike other ACADs which are homotetramers [10]. The protein sequence of ACAD9, however, does align with other human ACAD proteins, with 46–27% identity, and 56–38% similarity to eight other members of the ACAD family of proteins, with the highest similarity to VLCAD [26,32]. ACAD9 and the other ACAD family of proteins are associated with three domains of nearly equal size, with the N-terminal domain consisting of α-helices, the middle domain of a (5,8) barrel, and the C-terminal domain of a bundle of four helices [22]. The structure of the ACAD9 protein has been implicated in multiple roles, less importantly in fat metabolism and more in mitochondrial complex I gene regulation [25,26,32]. In a study by Nouws et al. 2010, the ACAD9 protein was found to directly bind and regulate the mitochondrial complex I gene regulation [25,26,32]. In a study by Nouws et al. 2010, the ACAD9 protein was found to directly bind and regulate the mitochondrial complex I gene regulation [25,26,32]. In a study by Nouws et al. 2010, the ACAD9 protein was found to directly bind and regulate the mitochondrial complex I gene regulation [25,26,32]. In a study by Nouws et al. 2010, the ACAD9 protein was found to directly bind and regulate the mitochondrial complex I gene regulation [25,26,32]. In a study by Nouws et al. 2010, the ACAD9 protein was found to directly bind and regulate the mitochondrial complex I gene regulation [25,26,32].

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## Table 1

| Author | Gender | Age | Mutation | Initial or most prominent symptoms | Age at onset | Blood lactate (mmol/L) |
|--------|--------|-----|----------|-----------------------------------|-------------|----------------------|
| He M et al. [17] | Male | Died at 14 years | TAAG insertion 44 bp upstream of first ATG | Refractory episodes, cerebellar stroke | 14 years | 10.8 |
| Female | Died at 10 years | Exon 3 deletion | N/A | Acute liver dysfunction, hypoglycemia | 4 months | N/A |
| Female | Died at 4.5 years | N/A | N/A | Cardiomyopathy with dilated left ventricle | 4.5 years | N/A |
| Haack TB et al. [15] | Female | Died at 46 days | F44I; R266Q | Cardiorespiratory depression, hypertrophic cardiomyopathy, encephalopathy, lactic acidosis | Birth | Elevated |
| Male | Died at 5 years | F44I; R266Q | Hypertrophic cardiomyopathy, mild exercise intolerance, persistent lactic acidosis | Birth | Elevated |
| Female | Died at 12 years | R266Q; R417C | Hypertrophic cardiomyopathy, encephalopathy, lactic acidosis | Birth | Elevated |
| Female | Died at 2 years | A326P; R532W | Hypertrophic cardiomyopathy, encephalopathy, lactic acidosis | Birth | Elevated |
| Dewulf et al. [9] | Female | Died at 5 months | N/A | Congenital cardiac and facial malformations, intractable pulmonary hypertension | Birth | 17.4 |
| Female | Died at 10.5 months | Homozygous V546L | Failure to thrive, colitis, recurrent infections, hypertrophic cardiomyopathy, lactic acidosis | 2 months | 20.8 |
| Male | Died at 9 months | Homozygous V546L | Failure to thrive, hypotonia, ulcerative colitis, hypertrophic cardiomyopathy, lactic acidosis | 15 days | 8.2 |
| Female | Died at 7 years | Homozygous V546L | Failure to thrive, recurrent infections, hypertrophic cardiomyopathy (dx at 4 years) | 15 months | N/A |
| Male | Died at 25 years | A170V; H563D | Growth retardation, exercise intolerance | 12 years | 12.48 |
| Female | Died at 22 years | A170V; H563D | Exercise intolerance, learning difficulty | 8 years | 4.42 |
| Female | Died at 11 months | R414S; L558X | Hypothrombopenia, hypoglycemia, lactic acidosis | Birth | 24.3 |
| Female | Died at 9 days | R414S; L558X | Right ventricular hypertrophy, other congenital cardiac defects, and lactic acidosis | Birth | 60.94 |
| Dewulf et al. [9] | Female | Died at 2 days | R414S; L558X | Hypertrophic cardiomyopathy, lactic acidosis | Birth | 25.27 |
| Garone et al. [12] | Male | Died at 13 years | Homozygous R414C | Psychomotor delay, proximal muscle weakness, generalized hypotonia, ataxic gait, bradykinesia and bradykinesia, scoliosis, and truncal obesity | 1 year | 10 |
| Female | Died at 15 years | Homozygous R532W | Easy fatigability, exercise intolerance, stroke like episode | After 4 years | 6.5 |
| Male | Died at 24 years | Homozygous R532W | Easy fatigability, exercise intolerance, stroke like episode | After 4 years | 2.7 |
| Leslie et al. [20] | Male | Died at 1 day | L314P; E63X | Respiratory distress, hypotonia, hepatomegaly, liver and cardiac failure | Birth | N/A |
| Female | Died at 8 months | Homozygous R518H | Failure to thrive, hepatomegaly, hypertrophic cardiomyopathy | 1 month | 7.6 |
| Female | Died at 11 months | Homozygous A220V | Hypertrophic cardiomyopathy, muscle weakness, hypotonia | 7 weeks | 20 |
| Our case | Female | Died at 6 months | V59F; L166W | Failure to thrive, dystonic posturing, neck and trunk hypotonia, microcephaly, lactic acidosis | 11 months | 5.4 |

### 5. Discussion

#### 5.1. Molecular genetics

The ACAD9 gene, localized on chromosome 3q21.3, consists of 18 exons [1,4]. The encoded ACAD9 protein is a 621 amino acid dimer with an altering α-helical architecture, unlike other ACADs which are homotetramers [10]. The protein sequence of ACAD9, however, does align with other human ACAD proteins, with 46–27% identity, and 56–38% similarity to eight other members of the ACAD family of proteins, with the highest similarity to VLCAD [26,32]. ACAD9 and the other ACAD family of proteins are associated with three domains of nearly equal size, with the N-terminal domain consisting of α-helices, the middle domain of a (5,8) barrel, and the C-terminal domain of a bundle of four helices [22]. The structure of the ACAD9 protein has been implicated in multiple roles, less importantly in fat metabolism and more in mitochondrial complex I gene regulation [25,26,32]. In a study by Nouws et al. 2010, the ACAD9 protein was found to directly bind and regulate NDUFAF1 and Ecsit, proteins specifically required for the assembly of mitochondrial complex I, arguably its most important role [26]. Upon entry into the mitochondria, ACAD9 undergoes a two step cleavage process, resulting in the removal of the first 37 amino acids, activating the enzyme [7,10,18]. ACAD9's catalytic activities include the oxidation of palmitoyl-CoA (C16:0) and stearoyl-CoA (C18:0), with three times higher activity of the former [32]. In a study by Nouws et al. 2014, catalytically inactive ACAD9 provided partial to complete rescue of complex I biogenesis in ACAD9 deficient cells, again highlighting its main role in complex I assembly [25].
To date 56 variants have been reported in the ACAD9 gene, with 16 determined to be pathogenic, 9 likely to be pathogenic, and 31 determined to be benign [2]. Prior to our report, the V59F and L166W variants had not been reported in the literature (Fig. 2). While no direct studies exist regarding these mutations, there are several lines of evidence that implicate these mutations as being pathogenic.

The expression profiles of 16 other mutations have been studied by Schiff et al. 2015 [30]. In this study, it was determined that mutations with little or no impact on ACAD9 activity and stability/folding were located after the C-terminal domain, while those which were inactivating were found in the catalytic portion of the molecule, which is conserved in all mitochondrial matrix ACADs (Fig. 2). Using a molecular modeling methodology developed by Nouws et al. 2010, the mutations in our patient were found to be localized at significant structural points for enzymatic activity, with amino acid 59 within the introductory portion of a peripheral α-helix (Fig. 3A), and amino acid 166 localized within an alpha helix near the catalytic site of the ACAD 9 protein (Fig. 3B) [26].

Based on their locations, mutations in these residues, as seen in our patient, likely result in significant alteration of the secondary and tertiary structure of the ACAD 9 protein, leading to functional deficits.

5.2. Phenotypic analysis

Mutations in the ACAD9 gene and the frequently associated mitochondrial complex I deficiency have resulted in multiple phenotypes of variable severity and onset (Table 1). The most common signs/symptoms include cardiac dysfunction due to hypertrophic cardiomyopathy. Our patient was found to have normal cardiac anatomy with clinically insignificant mild left ventricular hypertrophy which was discovered with subsequent screening after her diagnosis. Other common clinical findings include Leigh syndrome, macrocephaly, myopathies, and liver disease which our patient did not have [19,21,28]. The spectrum of symptom severity in patients with ACAD9 deficiency can range from mild exercise intolerance and easy fatigability in early childhood to acute liver and cardiac failure immediately after birth [13,20,31]. A common finding in essentially all patients reported in the literature is a lactic acidosis, indicating mitochondrial dysfunction, which was also found in our patient. Additionally, our patient’s muscle biopsy did have evidence of mitochondrial dysfunction on muscle electron transferring flavoprotein studies, however her biopsy did not show mitochondrial complex I deficiency, which is frequently associated with ACAD9 deficiency. Cardiac dysfunction due to hypertrophic cardiomyopathy, another feature strongly associated with mutations in the ACAD9 gene, presents variably, or rarely, completely absent, as seen in patients reported by Scholte et al. 1995 [9,13,31]. Our patient presented with normal cardiac anatomy with mild left ventricular hypertrophy and left ventricle mass greater than 95th percentile. While our patient did present with a mix of classic and unique ACAD9 deficiency findings, her other features add further to her uncommon ACAD9 phenotype.

The association between ACAD9 and Leigh syndrome, or subacute necrotizing encephalomyelopathy, has only recently been established in the literature and is due to the mitochondrial complex I deficiency caused by ACAD9 deficiency [20,29]. The pathophysiology of this disorder is due to the failure of the mitochondrial respiratory chain, caused...
by debilitating mutations in mitochondrial complex activity [27]. The syndrome itself is a heterogeneous, progressive, early-onset neurodegenerative disorder with regression of motor and mental skills leading to immobilization, retardation, and death [29]. It is likely that the degree of deficiency in ACAD9 enzymatic activity contributes to the severity of this syndrome, while mutations with high activity lead to less severe mitochondrial complex I deficiency, and a less devastating or altogether absent Leigh syndrome phenotype. Our patient, for example, did not present with Leigh syndrome.

Our patient's movement disorder is another example of her atypical phenotype. Choreathetoid movements have not been extensively described in patients with ACAD9 deficiency, but were a feature found distinctly in our patient. More commonly, hypotonia or exercise intolerance are expected in a patient with this condition [9,12,13,31]. A patient by Garone et al., 2013, had proximal muscle weakness, generalized hypotonia, ataxic gait, and bradykinesia [12]. While our patient also had neck and truncal hypotonia, her presenting symptoms of stiffening of the upper and lower extremities and a later onset of dystonic posturing, particularly with arching of the neck, are not described in the literature.

Microcephaly has not been reported in any case of ACAD9 deficiency. Macrocephaly is a more common presentation in these patients, likely in conjunction with the mitochondrial complex I deficiency that presents with this syndrome [15,28].

5.3. Treatment

Treatment for patients with ACAD9 deficiency includes both medications and supplements utilized for mitochondrial disorders, as well as for fatty acid oxidation disorder. Medium chain triglyceride (MCT) oil supplementation and a low fat, high protein diet, is important for long chain fatty acid (C14-C20) metabolism disorders, but the mainstay of treatment is riboflavin supplementation [13]. According to Haack et al.'s studies, supplementation with riboflavin leads to assembly and stability of the ACAD enzymes and therefore significant increase in mitochondrial complex I activity [15]. While certain mutations have been found to be resistant to riboflavin, the majority of ACAD9 deficiency cases can be partially treated with riboflavin supplementation [24]. Our patient's ambulation and visual fixation did improve with the addition of these therapies.

5.4. The role of whole exome sequencing (WES) in guiding diagnosis

The role of whole exome sequencing in guiding the diagnosis of ACAD9 deficiency in our patient was paramount. While muscle biopsy did suggest a mitochondrial pathology, no single ETC complex deficiency was identified, making determining the exact cause of her symptoms unlikely without the specificity of whole exome sequencing. Multiple studies suggest the importance of this modality for the diagnosis of disorders with complex phenotypes in the human population [8,11,14,23]. For a disorder such as ACAD9 deficiency which causes deficiency in both mitochondria and fatty acid oxidation, thereby presenting with multiple phenotypes, variable severity, and age of onset (Table 1), whole exome sequencing has been a revolutionary tool in guiding patients' diagnoses and thereby guiding their appropriate treatments [15].

6. Conclusion

This case illustrates a unique clinical presentation as well as the broad phenotypic variability of ACAD9 deficiency. The diagnosis of this disorder was not clinically suspected in this patient based on the symptomatology, lack of clinically significant cardiomyopathy, and mitochondrial findings not suggestive of complex I deficiency, cardinal features of this disorder.

Moreover, as an enzyme assay or analysis for ACAD9 has not been developed, the only and best diagnostic tool available was whole exome sequencing. WES provided the correct diagnosis for this patient, facilitating more specific, targeted medical treatment and thereby improving her clinical symptoms. The role of WES early in the evaluation of a patient with symptoms consistent with a mitochondrial disorder, may aid in expedited diagnosis and treatment, due to the overlap among this group of disorders that do not fit a “classic” phenotype. Our patient’s case is an example of the diagnostic utility and broadening of clinical knowledge that WES has provided.

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