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Exome sequencing in Jewish and Arabic patients with rhabdomyolysis reveals single-gene etiology in 43% of cases

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COMPLIANCE WITH ETHICAL STANDARDS

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Conflicts of interest The authors declare that they have no conflicts of interest.

Data availability statement All data generated or analyzed during this study are included in this published article.

WEB RESOURCES

1000 Genomes Browser, <http://browser.1000genomes.org>

ExAC Browser Beta, <http://exac.broadinstitute.org>

Exome Variant Server, <http://evs.gs.washington.edu/EVS/>

Genetics home reference site, <http://ghr.nlm.nih.gov/>

NCBI-gene, <http://www.ncbi.nlm.nih.gov/gene>

Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/omim>

Polyphen2, <http://genetics.bwh.harvard.edu/pph2/>

Pubmed, <http://www.ncbi.nlm.nih.gov/pubmed/>

UCSC Genome Browser, <http://genome.ucsc.edu>

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Abstract

Background—Rhabdomyolysis is a clinical emergency that may cause acute kidney injury (AKI). It can be acquired or due to monogenic mutations. Around 60 different rare monogenic forms of rhabdomyolysis have been reported to date. In the clinical setting, identifying the underlying molecular diagnosis is challenging, due to nonspecific presentation, the high number of causative genes, and current lack of data on the prevalence of monogenic forms.

Methods—We employed whole exome sequencing (WES) to reveal the percentage of rhabdomyolysis cases explained by single-gene (monogenic) mutations in one of 58 candidate genes. We investigated a cohort of 21 unrelated families with rhabdomyolysis, in whom no underlying etiology had been previously established.

Results—Using WES, we identified causative mutations in candidate genes in nine of the 21 families (43%). We detected disease-causing mutations in eight of 58 candidate genes, grouped into the following categories: 1) disorders of fatty acid metabolism (*CPT2*), 2) disorders of glycogen metabolism (*PFKM* and *PGAM2*), 3) disorders of abnormal skeletal muscle relaxation and contraction (*CACNA1S*, *MYH3*, *RYR1* and *SCN4A*), and 4) disorders of purine metabolism (*AHCY*).

Conclusions—Our findings demonstrate a very high detection rate for monogenic etiologies using WES and reveal broad genetic heterogeneity for rhabdomyolysis. These results highlight the importance of molecular genetic diagnostics for establishing an etiologic diagnosis. Because these patients are at risk for recurrent episodes of rhabdomyolysis and subsequent risk for AKI, WES allows adequate prophylaxis and treatment for these patients and their family members and enables a personalized medicine approach.

Keywords

Rhabdomyolysis; acute kidney injury; monogenic diseases; whole exome sequencing

INTRODUCTION

Rhabdomyolysis is a clinical emergency encountered by nephrologists. It is characterized by acute skeletal muscle damage, subsequent marked serum elevations in muscle enzymes (particularly creatine kinase (CK)), and increased risk for acute kidney injury (AKI). The differential diagnosis of rhabdomyolysis is heterogeneous [1]. In general, two types of underlying etiologic conditions have been reported. The first consists of acquired conditions such as trauma, infections, drugs and exposure to toxins. The second encompasses a high number of rare metabolic and neuromuscular monogenic disorders. In some cases, rhabdomyolysis may be secondary to a combination of genetic predisposition factors and environmental factors [1]. In those cases, the environmental factor may erroneously be

considered as the sole etiology, with the risk that the primary genetic etiologic diagnosis is missed and rhabdomyolysis episodes may recur [1].

In the clinical setting, identification of the exact underlying monogenic diagnosis is challenging, because more than 60 monogenic genes have already been implicated in rhabdomyolysis (Table 1) [1]. In addition, in most cases of rhabdomyolysis the clinical presentation is nonspecific including elevated CK, dark urine secondary to myoglobinuria, and AKI in some cases. To date, most of the available data regarding the genetic basis of rhabdomyolysis are based on individual gene-specific case reports. Therefore, no accurate estimates of monogenic etiology in patients presenting with rhabdomyolysis are known.

To address this issue, we investigated the frequency of disease-causing mutations in 21 unrelated individuals with rhabdomyolysis, who were evaluated in a tertiary children's hospital center in Israel between January 2012 and December 2015 by means of WES. We show that mutations in known rhabdomyolysis-causing genes are present in 43% of these families, and we outline important clinical implications generated from the knowledge of the molecular genetic diagnosis.

METHODS

Study participants

Following informed consent, we obtained clinical data, pedigree data, and blood samples from individuals with rhabdomyolysis from several medical centers in Israel. Approval for human subjects' research was obtained from the Institutional Review Boards of Sheba Medical Center, Boston Children's Hospital, and from other relevant local Ethics Review Boards. Informed consent was obtained from the individuals and/or parents, as appropriate. Included in the study were individuals with the diagnosis of rhabdomyolysis which was made by pediatric nephrologists and/or pediatric metabolists based on clinical presentation and elevated levels of CK. All patients presented or were referred for second opinion at Sheba Medical Center between January 2012 and December 2015. 21 out of 25 patients who presented during this time period were recruited to the study. All except one were children and or adolescents at presentation (under 21 years of age). There was only one case of adult who presented with rhabdomyolysis following general anesthesia, which he had for the first time in his life. He was referred to Safra children hospital (at Sheba Medical Center) as he was initially suspected to have glycogen storage disease for which the pediatric metabolic clinic in Safra Children's Hospital provides genetic analysis. This analysis was negative and given that he met inclusion criteria to the study this case was also included.

Whole exome sequencing (WES)

To identify the causative mutated gene for rhabdomyolysis, we investigated 21 unrelated index patients who were referred for second opinion in the outpatient clinics of Safra Children's Hospital due to rhabdomyolysis or had been admitted due to rhabdomyolysis to Safra Children's Hospital at Tel-Hashomer Medical Center in Israel. DNA samples from affected individuals were subjected to whole exome sequencing (WES) as established previously [2, 3] using Agilent SureSelect™ human exome capture arrays (Life

Technologies) with next generation sequencing (NGS) on an Illumina™ sequencing platform. Sequence reads were mapped against the human reference genome (NCBI build 37/hg19) using CLC Genomics Workbench (version 6.5.1) software (CLC bio). For homozygosity mapping, downstream processing of aligned BAM files was done using Picard and samtools [4]. SNV calling was performed using GATK [5] and the generated VCF file was subsequently used in homozygosity mapper [6]. Mutation calling was performed by geneticists and cell biologists, who had knowledge regarding clinical phenotypes, pedigree structure, genetic mapping, and WES evaluation and in line with proposed guidelines [7]. Sequence variants that remained after the WES evaluation process were examined for segregation.

Whole exome sequencing (WES) analysis

Following WES, all individuals' regions of homozygosity were identified. Genetic regions of homozygosity by descent ('homozygosity peaks') were plotted across the genome as candidate regions for recessive genes as previously described [3, 8]. Consanguinity was determined on the basis of long (>5 Mb) stretches of homozygosity. When homozygosity was present (three cases), we used homozygosity mapping in combination with WES as previously described [3, 8] to detect recessive homozygous disease-causing mutations. In one case, in which trio WES data was available to us, we performed trio analysis for homozygous as well as for compound heterozygous mutations. Finally, for all other individuals with no evidence of homozygosity by descent, we generated a list of previously reported rhabdomyolysis-causing genes and performed a targeted analysis for this predefined rhabdomyolysis gene panel.

Rhabdomyolysis gene panel

Previously reported rhabdomyolysis-causing genes were selected through a literature search in OMIM database, NCBI-gene, the Pubmed database, the Human Gene Mutation Database and the Genetics home reference site using the terms "rhabdomyolysis" and/or "myoglobinuria". Following review of the relevant reports, we included genes that have been implicated as an underlying monogenic cause for rhabdomyolysis. Altogether, 58 genes were selected (Table 1).

Variant calling

Following WES, genetic variants were first filtered to retain only non-synonymous and splice variants. Second, filtering was performed to retain only alleles with a minor allele frequency (MAF) <0.1% for dominant genes and <1% for recessive genes, widely accepted cutoffs for autosomal dominant and recessive disorders respectively [2, 8]. MAF was estimated using combined datasets incorporating all available data from the 1,000 Genomes Project, the Exome Variant Server (EVS) project, dbSNP142, and the Exome Aggregation Consortium (ExAC). Third, observed sequence variants were analyzed using the UCSC Human Genome Bioinformatics Browser for the presence of paralogous genes, pseudogenes, or misalignments. Fourth, we scrutinized all variants MAF<1% or <0.1% within the sequence alignments of the CLC Genomic Workbench™ software program for poor sequence quality and for the presence of mismatches that indicate potential false alignments. Fifth, we employed web-based programs to assess variants for evolutionary conservation, to

predict the impact of disease candidate variants on the encoded protein, and to determine whether these variants represented known disease-causing mutations. Sanger sequencing was performed to confirm the remaining variants in original DNA samples and when available to test for familial segregation of phenotype with genotype.

RESULTS

Our cohort included 21 unrelated individuals with rhabdomyolysis, and in whom the underlying etiology had not been established prior to this study. Most patients presented with no family history, additional symptoms or signs and without syndromic features (Table 2). Family history was reported only in four cases and only one case exhibited syndromic features in addition to rhabdomyolysis (Table 2).

Monogenic causes of rhabdomyolysis have been described for 58 genes (45 recessive, 7 dominant, 3 recessive or dominant, and 3 X-linked) and can be grouped into 7 categories (Table 1). We detected disease-causing mutations in 9 out of the 21 cases analyzed. In six cases the disease-causing mutations that we identified in five genes had been previously reported as pathogenic (*AHCY*, *CPT2*, *MYH3*, *PFKM* and *SCN4A*) [9–13]. In one case we found a novel mutation, which affects a residue that was previously shown to cause disease if altered (*RYR1*) [14, 15]. In two cases we identified highly conserved novel mutations (*CACNA1S* and *PGAM2*) (Table 2).

In two patients, ANX and AN3 (Table 2), we identified homozygous disease-causing mutations in *CPT2*. Mutations in *CPT2* lead to carnitine palmitoyltransferase II (CPT2) deficiency [10].

In two individuals, AN2 and AN5, we identified mutations in genes that cause two different forms of glycogen storage disease, *PFKM* and *PGAM2*. In *PFKM*, we identified a previously reported splice mutation [9] leading to Tarui Disease, also known as glycogen storage disease type VII. In *PGAM2* (Phosphoglycerate mutase 2 (Muscle)) we identified a highly conserved, novel homozygous missense mutation (Table 2). Mutations in this gene are responsible for an extremely rare autosomal recessive type of glycogen storage disease, also known as Glycogenosis type X [16].

In four individuals, we identified autosomal dominant mutations in genes that lead to rare disorders of abnormal skeletal muscle relaxation and contraction (*RYR1*, *CACNA1S*, *SCN4A*, and *MYH3*). Importantly, in two patients AN7 and AN11 we identified mutations in *RYR1* and *CACNA1S* (Table 2), which can lead to life-threatening malignant hyperthermia in the setting of certain environmental triggers such as exercise or drugs. AN13, in whom we identified a previously reported *SCN4A* pathogenic mutation (Table 2), presented with an autosomal dominant family history of rhabdomyolysis. Segregation analysis confirmed the presence of the pathogenic mutation in the patient's affected father. Mutations of the gene *SCN4A* lead to a skeletal muscle channelopathy with variable clinical presentation, including myotonia, episodic hypokalemic paralysis, and rhabdomyolysis in rare cases [17]. In patient AN21, we identified a previously reported pathogenic mutation in

MYH3 (Table 2) [12]. Mutations in this gene cause Freeman-Sheldon/Sheldon-Hall syndrome [12].

Finally, in one infant (AN20), who presented following birth with hypotonia, poor sucking reflex, apnea episodes and rhabdomyolysis, we identified two previously reported compound heterozygous pathogenic mutations in the gene *AHCY* (adenosylhomocysteinase) (Table 2) [13].

DISCUSSION

In the current study, we examined a group of 21 unrelated individuals with rhabdomyolysis for the presence of disease-causing mutations. We identified 8 different causative mutated genes in 9 out of 21 individuals (43%) studied. None of the above disease-causing mutations were suspected on clinical grounds prior to the current study, and affected patients were not clinically distinguishable from other rhabdomyolysis patients.

Our study has several limitations: First, in 11 individuals we did not identify underlying genetic etiology despite the fact that they presented similarly to the genetically solved cases. This, in part, can be explained by the known limitations of WES which does not detect copy number variations and mutations in non-coding regions. Those genetic abnormalities can theoretically affect one of the known rhabdomyolysis genes in those patients. In addition, it is also possible that there are still novel rhabdomyolysis genes that have not been characterized yet. Second, it is possible that the rhabdomyolysis cases we analyzed in this study may represent a relatively more severe fraction of patients and as such may lead to an over estimation of our disease causing mutations detection rate.

The causative mutated genes found in our cohort are grouped into four main categories (Table 1 and Table 2):

1. **Disorders of fatty acid metabolism:** mutations in *CPT2* are considered to be the most common monogenic cause for rhabdomyolysis [10, 18]. Mutations in *CPT2* lead to carnitine palmitoyltransferase II (*CPT2*) deficiency, a metabolic disorder of long-chain fatty-acid oxidation [18]. This syndrome has variable clinical presentations, which typically consist of one of the following three forms: 1) lethal neonatal form; 2) severe infantile hepato-cardio-muscular form; and 3) myopathic form. The myopathic form is characterized by exercise-induced muscle pain, rhabdomyolysis, myoglobinuria and as a result, predisposition to acute kidney injury. Other triggers include prolonged exercise (especially following fasting), cold exposure, infection, stress, sleep deprivation or general anesthesia. Patients usually will have no signs of myopathy or elevation of serum CK between attacks. Age at presentation can be variable ranging from the first to the sixth decade of life. While most individuals are mildly affected, severe renal failure requiring dialysis has been reported [19]. A definitive diagnosis of this syndrome can be made by detection of reduced CPT enzyme activity or by genetic testing of the *CPT2* gene. So far, more than 70 *CPT2* mutations have been reported. The p.Ser113Leu missense mutation that we identified in both patients accounts for 60% of cases of the myopathic CPT2

deficiency form and is considered to cause a milder phenotype within this group [20]. On the other hand, *CPT2* pathogenic null variants are often associated with the severe lethal neonatal form. Identification of the causative mutation will have consequences for the management of these patients; such as provision of a high-carbohydrate and low-fat diet in order to provide fuel for glycolysis. In addition, further episodes of rhabdomyolysis may be prevented by provision of glucose during infections to prevent catabolism, avoidance of extended fasting, avoidance of certain medications and refraining from prolonged exercise activity. Finally, providing adequate hydration during rhabdomyolysis episodes in order to prevent acute kidney injury is highly important.

2. **Disorders of glycogen metabolism:** the gene *PFKM* (Phosphofructokinase, muscle) encodes phosphofructokinase (PFK), which is the rate-limiting enzyme in the glycolytic pathway. It catalyzes the phosphorylation of fructose 6-phosphate to fructose-1,6-bisphosphate [21]. Mutations in this gene lead to glycogen storage disease type VII. Phenotypically, four different clinical presentations have been reported, including: (1) exercise intolerance, myalgias, and rhabdomyolysis; (2) a severe infantile form, with hypotonia, progressive myopathy, cardiomyopathy and respiratory failure; (3) a late onset form, with myopathy, usually appearing in the 5th decade and (4) a hemolytic form, characterized by hemolytic anemia without muscle symptoms [22]. PFK-deficient patients usually show a severe reduction of the enzyme activity in skeletal muscle and a partial deficiency in erythrocytes. Although the diagnosis can be made by testing the enzymatic activity, this has reported to be misleading in several cases, leaving the genetic diagnosis as the gold standard for definitive diagnosis [23]. So far, around 26 different disease-causing mutations have been described in the gene *PFKM*.

Deficiency of the terminal glycolysis enzyme, muscle phosphoglycerate mutase (PGAM), causes Glycogenosis type X, a metabolic myopathy characterized by exercise-induced cramps, rhabdomyolysis, and Myoglobinuria [16]. So far only eight different disease-causing mutations have been reported, mostly due to biallelic mutations. However, two independent reports also implicated a possible late onset symptomatic disease among heterozygote carriers [24, 25]. In the current study, we identified *PGAM* mutation in individual AN5 who presented at 20 years of age for evaluation of chronic symptoms of muscle cramps following exercise. Symptoms began at the age of 10 years, and were reported to appear even following moderate daily activities such as writing, climbing stairs, lifting objects, walking uphill or running. No similar symptoms were reported following fever or triggers other than physical exertion, and no changes were noted in urine color. The patient underwent extensive metabolic screening tests, including urine organic acids, serum acylcarnitine and Very Long Chain Fatty Acids (VLCFA) profiles which were all within normal limits. Echocardiogram, abdominal ultrasound, electromyography (EMG) and nerve conduction (NCV) tests were normal. In addition, a muscle biopsy (for light and electron microscopy) was performed elsewhere at the age of 12 years and was reported as normal,

including normal respiratory chain enzymes. A forearm ischemic test showed elevation of serum ammonia (from 46.3 mcg/dl at baseline to 651.4 mcg/dl; N 31-123) and lactate levels (from 3.9 mg/dl to 13.5 mg/dl; N 6-18). Past genetic testing included targeted sequencing for known mutations in CPT2 for which the patient was negative. Family history was notable for a paternal uncle and grandfather which had both experienced occasional muscle cramps without related functional disability. The PGAM2 mutation we identified in this patient was never reported in homozygous state in all available public data bases, was predicted to be deleterious by 3 different prediction programs (SIFT, mutation taster and Polyphen), was located in one of the protein's domains (Phosphoglycerate mutase 1) and affected a highly conserved residue. Continuous evolutionary conservation of the amino acid residue was evident across the following species: *Mus musculus*, *Gallus gallus*, *Xenopus tropicalis*, *Danio rerio*, *Ciona intestinalis*, *Caenorhabditis elegans* and *Drosophila*.

3. **Disorders of abnormal skeletal muscle relaxation and contraction:** malignant hyperthermia could be caused by mutations in *RYR1* and *CACNA1S*. More than 500 different mutations in *RYR1*, encoding the Ryanodine receptor 1, have emerged as the underlying cause of a wide clinical spectrum encompassing periodic exertional rhabdomyolysis and severe life-threatening malignant hyperthermia [26]. Ryanodine receptor 1 is an ion channel responsible for the release of calcium from the sarco/endoplasmic reticulum [26]. It functions in concert with the α -subunit of the voltage-dependent L-type calcium channel, encoded by *CACNA1S* [26]. *CACNA1S* is important for the activation of RYR1 and for which approximately 30 mutations have been reported to date. In the current study we identified RYR1 mutation in individual AN7 and *CACNA1S* mutation in individual AN11. AN7, a 46 year old male presented for evaluation following elevated CK levels (reaching 93,000 U/L) obtained due to dark colored urine following general anesthesia for an elective inguinal hernia repair. The patient was born to non-consanguineous parents of Ashkenazi-Jewish descent, and his family history was non-contributory. His father and two brothers were reported to be asymptomatic, and had never required general anesthesia. Of note, the patient reported of regular exercise as a volleyball player, after which he remains asymptomatic. He did not undergo muscle biopsy, and was sent directly for whole exome sequencing. He was found to harbor a novel highly conserved missense mutation in RYR1 (c.179A>G; p.Asp60Gly). This mutation affects an amino acid residue that was previously shown multiple times to cause disease if altered [14, 15, 27]. As parental DNA was not available to us, the question whether this mutation was de novo or not needs to remain open, in this disease where there can be incomplete penetrance.

AN11, currently 23 years old, presented at 20 years of age for evaluation due to myalgia and abdominal pain accompanied by elevation of serum CK levels to 17,974 U/L. Symptoms were preceded by an episode of extreme and exercise as part of his military training. He was afebrile and no change was noted in urine color. His family history was reportedly negative; however his two older siblings

have never undergone similar strenuous physical exertion. Previous medical history included a single episode of mild CK elevation to 800 U/L following pneumonia. Genetic evaluation prior to the WES included Sanger sequencing for known CPT2 mutations common in the Ashkenazi-Jewish population, for which he was found negative. This patient was found to harbor a novel highly conserved mutation in CACNA1S. This mutation was never reported in any available data bases including the ExAC and is predicted to be deleterious (max PolyPhen-2 (PP2) humvar score of functional “deleteriousness” of 1.0). In addition, the mutation is located in ion transport domain of the protein and affects a highly conserved residue. Continuous evolutionary conservation of the amino acid residue was evident across the following species: *Mus musculus*, *Gallus gallus*, *Xenopus tropicalis*, *Danio rerio*, *Ciona intestinalis*, *Caenorhabditis elegans* and *Drosophila*.

The gene *SCN4A* encodes the α -subunit of the skeletal muscle voltage-dependent sodium channel type 4, and mutations in *SCN4A* lead to a skeletal muscle channelopathy with variable clinical presentation, including myotonia, episodic hypokalemic paralysis, and rhabdomyolysis in rare cases [17]. Interestingly, mutations in *MYH3* usually lead to a rare and severe form of arthrogryposis and myopathy also known as Freeman-Sheldon/Sheldon-Hall syndrome [12]. Mild clinical presentations, as well as rhabdomyolysis, have been reported in this syndrome [28].

4. **Disorders of purine metabolism:** only a few families have been reported to harbor mutations in the gene *AHCY*, which encodes the enzyme S-adenosylhomocysteine (SAH) hydrolase. This enzyme catalyzes the final step in the conversion of methionine to homocysteine. Disease severity and phenotype can be variable; however, most affected individuals exhibit delayed psychomotor development and hypotonia at birth, accompanied by elevated serum aminotransferase [29]. Elevated levels of muscle enzymes such as CK, LDH, and AST have been also reported in a few cases [30]. Importantly, dietary methionine restriction and dietary supplements of creatine and phosphatidylcholine can substantially lower circulating levels of methionine in SAH hydrolase-deficient patients [29, 31], though its long-term clinical benefits are still unknown.

Our study highlights several important insights for the diagnosis of rhabdomyolysis: First, it demonstrates a high prevalence of monogenic etiologies for individuals with rhabdomyolysis, which in most part was underappreciated. The fact that most cases presented sporadically without a positive family history led to a low index of suspicion for the genetic nature of this condition. As a result, several cases were falsely assigned only to be secondary to environmental exposures such as exertion or drug intake. As both features play a role, exertion as well as monogenic mutations, this disease group gives an example of monogenic disorders in the context of the gene-environment interaction. Second, individuals with mutations in genes that cause rhabdomyolysis can initially present with kidney-related symptoms and signs of acute kidney injury and/or dark urine. As a result, nephrologists, who may be the first clinicians to be approached, should be aware that there are over 60 different syndromes that can have rhabdomyolysis as one of their features in those patients. Third, our

results suggest broad genetic heterogeneity for rhabdomyolysis. To the best of our knowledge, only few rhabdomyolysis cohorts were systematically analyzed by means of next generation sequencing and or whole exome sequencing [32–35]. Given the rarity and the high number of genes that can cause rhabdomyolysis if mutated, WES should be the genetic method of choice for molecular diagnosis. In this setting, WES offers a powerful, cost effective, and non-invasive tool for a precise, unequivocal, etiology-based diagnosis. Most importantly, the genetic molecular diagnosis enabled us to provide personalized therapeutic management as well as to alert from possible life-threatening events such as malignant hyperthermia and acute kidney injury, which can be caused by mutations of some of the rhabdomyolysis genes.

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Table 1

Fifty-eight monogenic causes of rhabdomyolysis. (The eight genes, in which mutations were found in this study are underlined.)

Gene	MOI	Disease name	Reference (PMID)
1) Disorders of fatty acid metabolism			
<i>ACADM</i>	AR	Medium-chain acyl-CoA dehydrogenase (MCAD) deficiency	(7876853)
<i>ACADS</i>	AR	Short-chain acyl-CoA dehydrogenase (SCAD) deficiency	(24946698, 16531950)
<i>ACADVL</i>	AR	Very long-chain acyl-CoA dehydrogenase (VLCAD) deficiency	(24263034, 8145917)
<u><i>CPT2</i></u>	AR	Carnitine palmitoyltransferase II (CPT II) deficiency	(21314018, 15363638)
<i>ETFA</i>	AR	Multiple acyl-CoA dehydrogenase deficiency	(25200064)
<i>ETFB</i>	AR	Multiple acyl-CoA dehydrogenase deficiency	(25200064)
<i>ETFDH</i>	AR	Multiple acyl-CoA dehydrogenase deficiency	(25200064, 22041377)
<i>SLC22A5</i>	AR	Primary carnitine deficiency	(24946698)
<i>SLC25A20</i>	AR	Carnitine-acylcarnitine translocase deficiency	(24088670)
2) Disorders of glycogen metabolism			
<i>ALDOA</i>	AR	Glycogen storage disease type XII	(25392908)
<i>ENO3</i>	AR	Glycogen storage disease type XIII	(25267339)
<i>LDHA</i>	AR	Glycogen storage disease type XI (also known as Lactate dehydrogenase deficiency)	(22127970)
<u><i>PFKM</i></u>	AR	Glycogen storage disease type VII (also known as Tarui disease)	(18421897)
<u><i>PGAM2</i></u>	AR	Glycogen storage disease type X	(16881065, 19783439)
<i>PGK1</i>	XLR	Phosphoglycerate kinase deficiency	(6830158, 7082849)
<i>PGM1</i>	AR	Phosphoglucomutase 1 deficiency	(24499211)
<i>PHKA1</i>	XLR	Glycogen storage disease type IX	(12825073, 9731190)
<i>PHKB</i>	AR	Glycogen storage disease type IX	(25929793)
<i>PYGM</i>	AR	Glycogen storage disease type V (also known as McArdle disease)	(18833216, 25293680)
3) Mitochondrial disorders			
<i>ACAD9</i>	AR	Mitochondrial complex I deficiency due to acyl-CoA dehydrogenase 9 deficiency	(17564966)
<i>BCS1L</i>	AR	Mitochondrial complex III deficiency	(11528392)
<i>DGUOK</i>	AR	Deoxyguanosine kinase deficiency	(23043144)
<i>DLD</i>	AR	Dihydrolipoamide dehydrogenase deficiency (also known as Maple syrup urine disease type 3)	(9040667)
<i>FDX1L</i>	AR	Mitochondrial myopathy	(24281368)
<i>HADHA</i>	AR	Mitochondrial trifunctional protein deficiency	(9739053, 21549624)
<i>HADHB</i>	AR	Mitochondrial trifunctional protein deficiency	(12754706)
<i>ISCU</i>	AR	Myopathy with deficiency of iron-sulfur cluster assembly enzyme	(20206689)
<i>POLG</i>	AD/AR	POLG-related mitochondrial diseases	(23873972, 9443501)
<i>TSMF</i>	AR	Combined oxidative phosphorylation deficiency 3	(17033963)
4) Disorders of purine metabolism			
<i>ADSL</i>	AR	Adenylosuccinate lyase deficiency	(24946698)

Gene	MOI	Disease name	Reference (PMID)
<i>AMPD1</i>	AR	Adenosine monophosphate deaminase deficiency	(8335021)
<i>AHCY</i>	AR	Hypermethioninemia with deficiency of S-adenosylhomocysteine hydrolase	(16736098, 15024124)
5) Myopathies			
<i>ANO5</i>	AR	Anoctaminopathy-5	(24889862)
<i>CASQ1</i>	AD	Vacuolar myopathy with CASQ1 aggregates	(25116801)
<i>CAV3</i>	AD/AR	Caveolinopathy	(27312022)
<i>DMD</i>	XLR	Duchenne and Becker muscular dystrophy	(19762730)
<i>DYSF</i>	AR	Dysferlinopathy	(22550092)
<i>FKRP</i>	AR	Muscular dystrophy-dystroglycanopathy	(22029705)
<i>FKTN</i>	AR	Muscular dystrophy-dystroglycanopathy	(6498017)
<i>GMPPB</i>	AR	Muscular dystrophy-dystroglycanopathy	(25681410)
<i>ISPD</i>	AR	Muscular dystrophy-dystroglycanopathy	(23390185)
<i>SGCA</i>	AR	Alpha-sarcoglycanopathy	(26453141, 23989969)
6) Disorders of abnormal skeletal muscle relaxation and contraction			
<i>ATP2A1</i>	AR	Brody myopathy	(25614869)
<i>CACNA1S</i>	AD	Malignant hyperthermia susceptibility 5, Hypokalemic periodic paralysis	(26238698, 9199552, 25658027)
<i>MYH3</i>	AD	Freeman-Sheldon syndrome, Sheldon-Hall syndrome	(16642020, 16510655, 24431877)
<i>RYR1</i>	AD/AR	Malignant hyperthermia susceptibility 1, Exertional rhabdomyolysis, Congenital myopathies	(23628358, 23476141)
<i>SCN4A</i>	AD	Hyperkalemic periodic paralysis type 2, Hypokalemic periodic paralysis type 2, Potassium-aggravated myotonia, Paramyotonia congenita	(23801527)
7) Miscellaneous			
<i>AMACR</i>	AR	Alpha-methylacyl-CoA racemase deficiency	(20921516)
<i>CTDP1</i>	AR	Congenital cataracts, facial dysmorphism, and neuropathy	(20301787)
<i>CYP2C8</i>	AR	Cerivastatin-induced rhabdomyolysis	(15365880)
<i>HMBS</i>	AD	Acute Intermittent Porphyria	(25389600, 18647325)
<i>HRAS</i>	AD	Costello syndrome	(8834040)
<i>KCNJ11</i>	AR	Congenital hyperinsulinism	(24421282)
<i>LPIN1</i>	AR	Myoglobinuria, acute recurrent, autosomal recessive	(20583302)
<i>QARS</i>	AR	Microcephaly, progressive, with seizures and cerebral and cerebellar atrophy	(24656866)
<i>SIL1</i>	AR	Marinesco-Sjögren syndrome	(9638664)
<i>SLC16A1</i>	AD	Erythrocyte lactate transporter defect	(3775384)
<i>TSEN54</i>	AR	Pontocerebellar hypoplasia type 2	(23177318)

Abbreviations: AD, autosomal dominant; AR, autosomal recessive; MOI, mode of inheritance; PMID, Pubmed ID; XLR, X-linked recessive.

Table 2

Disease causing mutations and clinical phenotypes in 9/21 individuals presenting with rhabdomyolysis.

Indiv- idual	Origin	Causative gene (Syndrome name, MOI)	Nucleotide alteration	Alteration in coding sequence*	Zygo- sity	Conti- nuous AA- sequence Conser- vation	Presenting symptoms (Age; and CK level [U/L] at presentation)	Family history of rhabdomyolysis	Highest creatinine (mg/dL)
ANX	Ashkenazi Jewish	<i>CPT2</i> (Carnitine palmitoyltransfe- rase II deficiency, AR)	c.338C>T	p.Ser113Leu	Hom	<i>Dm</i>	Muscle cramps, myoglobinuria following exercise (16y; 160,000)	No	0.9
AN3	Arab	<i>CPT2</i> (Carnitine palmitoyltransfe- rase II deficiency, AR)	c.338C>T	p.Ser113Leu	Hom	<i>Dm</i>	Fever and muscle cramps (9y; 130,000)	No	0.76
AN2	Ashkenazi Jewish	<i>PFKM</i> (Tarui disease, AR)	c.237+1G>A	splice	Hom	<i>Splice</i>	Fatigue and weakness following exercise (4y; 1,801) Muscle cramps	Sister showed similar clinical and laboratory presentation Parents are healthy.	N/A
AN5	Iranian Jewish	<i>PGAM2</i> (Glycogen storage disease X, AR)	c.637G>A	p.Gly213Arg	Hom	<i>Dm</i>	following exercise (10y; 2,000) Dark colored	Paternal aunt has rarely muscle weakness.	0.66
AN7	Ashkenazi Jewish	<i>RYR1</i> (Malignant hyperthermia susceptibility 1, AD)	c.179A>G	p.Asp60Gly	Het	<i>Dr</i>	urine and CPK elevation following general anesthesia (46y; 93,000)	No	N/A
AN11	Ashkenazi/Bukharian Jewish	<i>CACNA1S</i> (Malignant hyperthermia susceptibility 5, AD)	c.1678G>T	p.Ala560Ser	Het	<i>Dm</i>	Myalgia, abdominal pain following extreme exercise (20y; 17,974)	No	1.16
AN13	Greek Jewish	<i>SCN4A</i> (Hyperkalemic periodic paralysis, type 2, AD)	c.4343G>A	p.Arg1448His	Het	<i>Dr</i>	Abdominal pain, general weakness and fatigue (20y; 870) Myalgia and	Patients' sibling, father and paternal uncle had elevated CK level following exercise. Maternal	1.2
AN21	Ashkenazi Jewish	<i>MYH3</i> (Freeman- Sheldon syndrome, AD)	c.875C>G	p.Ser292Cys	Het	<i>Dr</i>	paresthesia (15y; 24,000)	grandfather with elevated CK	0.64
AN20	Sephardic Jewish	<i>AHCY</i> (Hypermethionin- emia with deficiency of S- Adenosylhomo- cysteine hydrolase, AR)	c.266C>T c.428A>G	p.Ala89Val p.Tyr143Cys	Het Het	<i>Dm Ce</i>	Hypotonia, poor sucking reflex, apneas (2d; 12,353)	No	0.3

* All mutations had a PolyPhen2 (PP2) humvar score of functional 'deleteriousness'.

Abbreviations: AA, amino acid; AD, Autosomal dominant; AR, autosomal recessive; CK, creatine kinase; *Ce*, *Caenorhabditis elegans*; *Dm*, *Drosophila melanogaster* ; *Dr*, *Danio rerio*; d, day; Het, heterozygous; Hom, homozygous; MOI, mode of inheritance; N/A, not applicable; y, year.