CLINICAL AND MOLECULAR-GENETIC PROFILES OF PATIENTS WITH MORPHOLOGICAL INDICATIONS OF CONGENITAL MULTICORE MYOPATHY

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CONGENITAL CORE MYOPATHIES ARE A CLINICALLY AND GENETICALLY HETEROGENEOUS GROUP OF CONGENITAL MYOPATHIES THAT SHARE A SPECIFIC HISTOPATHOLOGICAL FEATURE: AREAS OF REDUCED OXIDATIVE ACTIVITY IN MUSCLE FIBERS. THE RELATIONSHIP BETWEEN CLINICAL, GENETIC AND MORPHOLOGICAL CHARACTERISTICS OF THIS GROUP OF DISORDERS REMAINS UNDERSTUDIED. THE AIM OF THIS WORK WAS TO COMPARE CLINICAL PRESENTATIONS AND MORPHOLOGICAL PHENOTYPES OF PATIENTS WITH CONGENITAL MYOPATHIES/MYODYSTROPHY TO THE DATA YIELDED BY MASSIVELY PARALLEL EXOME SEQUENCING. EIGHT PARTICIPANTS HAD SEVEN MUTATIONS IN GENES ASSOCIATED WITH CONGENITAL CORE MYOPATHIES; ONE PATIENT HAD 2 MUTATIONS IN THE LAMA2 GENE IMPLICATED IN MEROSIN-DEFICIENT MUSCULAR DYSTROPHY. THE PROPORTIONS OF PATIENTS WITH MUTATIONS IN RYR1 AND SEPN1 WERE EQUAL (42.86%). OF 10 DETECTED MUTATIONS, 3 HAD NOT BEEN PREVIOUSLY DESCRIBED, INCLUDING c.7561G>A IN RYR1, c.485C>A IN SEPN1 AND p.Cys1136Arg IN LAMA2. THE CLINICAL AND MORPHOLOGICAL FEATURES OF CORE MYOPATHIES SUGGEST THAT GENETIC CAUSES OF THIS GROUP OF DISORDERS SHOULD NOT BE LIMITED ONLY TO RYR1 AND SEPN1 GENES ONLY. THIS NECESSITATES THE SEARCH FOR AND THE STUDY OF OTHER GENES IMPLICATED IN CONGENITAL MYOPATHIES OR MYODYSTROPHY USING STATE-OF-THE-ART MOLECULAR GENETIC TOOLS.

Keywords: congenital central core disease, congenital multicore myopathies, RYR1 gene, SEPN1 gene, LAMA2 gene, muscle biopsy, exome sequencing

Author contribution: Kozina AA — literature analysis, analysis and interpretation of exome sequencing data, manuscript preparation; Shatalov PA — data acquisition, microscopy, manuscript preparation; Baranich TI — microscopy; Artemieva SB — clinical data acquisition; Baryshnikova NV — literature analysis, analysis and interpretation of exome sequencing data, manuscript preparation; Krasnenko AYu — exome sequencing; Ilinsky VV — exome sequencing; Sukhorukov VS — study design, data acquisition

Compliance with ethical standards: the study was approved by the Ethics Committee of Pirogov Russian National Research Medical University (Protocol № 172 dated February 19, 2018). All participants or their legal representatives gave informed consent to participate.

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Received: 07.02.2019 Accepted: 19.04.2019 Published online: 30.04.2019
DOI: 10.24075/brsmu.2019.034

КЛИНИЧЕСКИЕ И МОЛЕКУЛЯРНО-ГЕНЕТИЧЕСКИЕ ХАРАКТЕРИСТИКИ ПАЦИЕНТОВ С МОРФОЛОГИЧЕСКОЙ КАРТИНОЙ ВРОЖДЕННОЙ СТЕРЖНЕВОЙ МИОПАТИИ

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Врожденные стержневые миопатии — это клинически и генетически гетерогенная группа врожденных миопатий, общий гистопатологический признак которых — наличие участков с уменьшенной окислительной активностью при биопсии мышц. Взаимосвязь клинико-генетических, патогенетических и морфологических характеристик этой группы миопатий до конца не изучена. Целью исследования было проанализировать соответствие клинико-морфологических характеристик пациентов с врожденными миопатиями/миодистрофиями и результатов экзомного секвенирования, полученных методами массивового параллельного секвенирования (MPS).

В исследовании участвовали 8 детей (2 мальчика и 6 девочек, 3–14 лет). Морфологический анализ проводили с помощью световой и электронной микроскопии. Молекулярно-генетический анализ проводили с помощью MPS на платформе HiSeq2500. Мутации были обнаружены в 87,5% случаев (у 7 из 8 обследованных): у 6 обследованных (8 мутаций) — в генах, ответственных за врожденные стержневые миопатии, и у одного пациента (2 мутации) — в гене LAMA2, ответственном за меросин-негативную мышечную дистрофию. Доля патологий с выявленными мутациями в гене RYR1 и мутациях в гене SEPN1 одинакова и составила 42,86% среди пациентов с мутациями. Из 10 мутаций, выявленных у обследованных пациентов, 3 мутации описаны впервые: в гене RYR1 — с.7561G>A; в гене SEPN1 — с.485C>A; в гене LAMA2 — p.Cys1136Arg. Совокупность клинических и морфологических признаков, характерных для стержневых миопатий, не позволяет ограничить молекулярно-генетический поиск причины заболевания генами RYR1 и SEPN1, что приводит к необходимости исследовать другие гены, ответственные за развитие врожденных миопатий/миодистрофий, с использованием современных молекулярно-генетических методов.

Ключевые слова: врожденные миопатии центрального стержня, врожденные многостержневые миопатии, ген RYR1, ген SEPN1, ген LAMA2, мышечная биопсия, экзомное секвенирование

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Соблюдение этических стандартов: исследование одобрено этическим комитетом ФГБОУ ВО РНМУ имени Н. И. Пирогова (протокол № 172 от 19 февраля 2018 г.). Выполнение всех требований в соответствии с законодательством Российской Федерации по обеспечению конфиденциальности, анонимности и ненарушения прав и свобод участников исследования.

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Статья получена: 07.02.2019
Статья принята к печати: 19.04.2019
Опубликована онлайн: 30.04.2019
DOI: 10.24075/brsmu.2019.034
Congenital myopathies constitute a clinically and genetically heterogeneous group of neuromuscular disorders with complex pathogenesis, diverse symptoms and different inheritance patterns [1]. In the affected patients, the loss of muscle function is a result of structural changes of muscle fibers in the absence of dystrophic changes in muscle tissue [2].

There are a few classic forms of congenital myopathies that can be distinguished histologically, the most common being central core disease, nemaline myopathy, multiminicore myopathy, and centronuclear myopathy [2].

Each of these forms has a number of genetic subtypes differing in the severity of presenting symptoms and the patterns of inheritance. This should be accounted for when deciding on an adequate treatment strategy or providing genetic counseling.

Because some congenital myopathies are quite rare, genetically heterogeneous and share symptoms with other neuromuscular disorders, their differential diagnosis and accurate classification pose a challenge, making it difficult to estimate the actual prevalence of these subtypes and their morphological phenotypes or to assess their contribution to the floppy baby syndrome. The limitations of existing sequencing methods complicate the situation even further.

Congenital myopathies have been studied since 1956 when central core disease was first described. However, the etiopathogenesis and the associations between clinical presentations and genetic phenotypes of congenital core myopathies are still not fully clear.

Morphologically, all core myopathies are characterized by areas of reduced oxidative enzyme activity in type 1 muscle fibers, sarcomere disorganization and almost complete depletion of mitochondria visible during a histochemical examination [3, 4]. Core myopathies can be broken down into two major types: central core disease and multiminicore myopathy.

According to the literature, hereditary core myopathies are largely caused by mutations in the genes coding for two proteins of the sarcoplasmic reticulum: the ryanodine receptor (RYR1) and selenoprotein N (SEPN1) [5, 6].

The ryanodine receptor gene (RYR1) is located on chromosome 19q13.1 and comprises 106 exons. The receptor itself is a calcium release channel of the endoplasmic reticulum membrane. In skeletal muscles, this receptor is embedded in the sarcoplasmic reticulum membrane, where it interacts with the dihydropyridine receptor located on plasma membrane invaginations known as transverse tubules. Electrical signals that travel along the sarcolemma activate calcium release from the sarcoplasmic reticulum following the coupling of the two receptors, and the muscle contracts [7–9]. Mutations tend to occur more often in the ryanodine receptor gene (RYR1) than in the gene coding for selenoprotein N (SEPN1). Mutant RYR1 variants are associated with a few pathological conditions, such as an autosomal dominant or autosomal recessive central core disease, in the first place (OMIM entry #117000), and, less often, multiminicore disease (OMIM entry #255320) with an autosomal pattern of inheritance [10, 11]. Mutations in RYR1 are associated with an increased susceptibility to autosomal dominant malignant hyperthermia (OMIM entry #145600), a predisposition to severe and potentially lethal adverse reactions to volatile anesthetics and/or muscle relaxants [12].

Central core disease is driven by ultrastructural changes and the loss of enzyme activity (exerted by mitochondrial enzymes, in particular) in the center of skeletal muscle fibers. Histologically, these “cores” differ from the peripheral areas of the fibers, so their morphology is the major diagnostic criterion for this disorder [13] (Fig. 1 and 2). Central core disease usually has an onset in early infancy. Among its typical symptoms are motor development delay, low muscle tone, and weakness of proximal muscles (facial muscles are spared). Skeletal manifestations are not rare, including congenital hip dislocation and scoliosis. Hypotonia does not progress with age.

The SEPN1 gene is located on chromosome 1p36-p35, comprises 13 exons and codes for selenoprotein N, a glycoprotein of the endoplasmic reticulum and a selenium mediator. Selenoprotein is an important component of many metabolic pathways and antioxidant systems. It also helps to maintain calcium homeostasis in muscle tissue by stimulating oxidative enzymes and regulating the oxidative state of ryanodine receptors. Deficit in selenoprotein N promotes oxidation in myotubes and entails deregulation of superoxide dismutase and catalase. This causes oxidative stress; dysfunctional ryanodine receptors can no longer control calcium release from the endoplasmic reticulum, disrupting calcium homeostasis in muscle tissue [9, 11, 14]. Mutations in SEPN1 are associated with multicore myopathies (OMIM entry #602771) and congenital rigid spine muscular dystrophy (OMIM entry #602771), which has a similar phenotype [15]. The inheritance pattern here is autosomal recessive.
Histologically, multicores myopathies are characterized by the presence of multiple cores in skeletal muscle fibers that do not necessarily affect the center of the fiber (Fig. 3). In RYR1-associated multinicromeic myopathy, the cores are quite massive [9, 11]. In SEPN1-associated myopathy, multicores are abundantly present in the muscle tissue [9, 11].

Multinicromeic myopathy is a congenital myopathy that develops in infancy and manifests itself as a floppy baby syndrome. The symptoms include low muscle tone, delayed motor development, proximal muscle weakness, spinal deformities, early onset of scoliosis, and chest deformities. Facial muscle weakness is also typical.

The similarity of clinical presentations between core myopathies and other congenital myopathies, the complexity of these diseases, and the existence of different combinations of muscle tissue abnormalities complicate the differential diagnosis. The diagnosis of congenital myopathy is based on the assessment of both clinical and morphological presentations and the results of molecular-genetic tests. Patients with structural myopathies do not always share the same clinical and morphological phenotype. Although such myopathies are linked to the mutations in the RYR1 and SEPN1 genes, there is a hypothesis suggesting that other genes may also be involved; some authors also hypothesize the polygenic nature of these neuromuscular conditions [2]. In light of this, it may be relevant to explore clinical, morphological and genetic aspects of congenital core myopathies in parallel.

The aim of this study was to compare clinical and morphological features and the results of massively parallel exome sequencing in patients with clinical symptoms and histological evidence of congenital core myopathies.

METHODS

The study was carried out in 8 children (2 boys and 6 girls) aged 3 to 14 years. The study included patients of both sexes with no family history of neurological disorders who were diagnosed with congenital myopathy and whose histological samples suggested core myopathy; the patients also underwent molecular genetic testing. The following exclusion criteria were applied: the absence of morphological signs of core myopathies in biopsy samples.

The patients’ medical records were analyzed. Biopsy samples were studied by light and electron microscopy following the protocols supplied by the manufacturers. Prior to light microscopy, the samples were either paraffinized or frozen and then stained.

1. Fresh tissue sections were prepared by immersing the samples in liquid nitrogen and then slicing them on a Microm HM 505 N cryostat microtome (Microm Tech.; USA). Paraffin sections were prepared by fixing biopsy material in 10% neutral formalin following the manufacturer’s protocol and then slicing it on the microtome.

2. Hematoxylin-eosin staining of both frozen and paraffinized sections of the examined muscle fibers (the arrows)


RESULTS

The patients had similar clinical symptoms typical of congenital structural myopathies: the floppy baby syndrome in infancy and delayed motor development. Gait was also affected; some patients were unable to walk on their own; spinal deformities were observed. Neurological symptoms included low muscle tone and strength, weak or absent tendon reflexes in the upper and lower limbs. Biochemistry tests revealed normal creatine

Fig. 3. Skeletal muscle tissue of a 3.5-year-old patient with congenital multinicromeic myopathy. Histochemical analysis of succinate dehydrogenase activity in frozen sections (x200; the method was proposed by Sukhorukov VS using the nitro BT method by Nachlass et al. [19]). Cores are abundant in almost all longitudinal sections of the examined muscle fibers (the arrows)
phosphokininase (CPK) levels in 3 children under 5 years of age; in 5 children aged 7 to 14 years CPK was either high but within the reference range or slightly elevated above the norm (Table 1). Cores were observed in the biopsy samples of 5 patients (patients 1, 2, 3, 7, and 8) diagnosed with central core disease; histological analysis suggested multiminicore myopathy in 3 patients (patients 4, 5, and 6).

Mutations were detected in 87.5% of cases (7 of 8 examined patients). Six children had mutant variants of RYR1 and SEPN1 implicated in congenital myopathy; One patient had a mutation in LAMA2 associated with merosin-deficient muscular dystrophy (Table 2).

DISCUSSION

Sequencing data are well-correlated with the clinical presentations and morphological features of the patients: in 3 patients with a morphological phenotype of central core disease (patients 1, 2 and 3), mutations were observed in gene RYR1; 3 patients with a morphological phenotype of multicore myopathy (patients 4, 5 and 6) had mutations in gene SEPN1 (Table 2). The proportions of patients with mutations in RYR1 and SEPN1 were equal and amounted to 42.86 % each relative to all the examined patients with mutations.

Three patients had 4 mutations in RYR1. Of those mutant variants, 3 were not described previously, including c.11798A>G, c.14387A>G and c.14581C>T [21, 22, 23].

According to the literature, mutations c.11798A>G and c.14387A>G are associated with sporadic central core disease; our patients who carried them (patients 1 and 2, respectively) were also heterozygous.

Patient 3 had 2 mutations (c.14581C>T and c.7561G>A) in the RYR1 gene that were presumably compound heterozygous, which suggests an autosomal recessive pattern of inheritance. Mutation c.14581C>T is known to occur in patients with sporadic central core disease but can also be recessive [27]. Mutation c.7561G>A was not described previously. The presence of 2 mutations in one patient suggests an autosomal recessive pattern of inheritance.

Three patients had 4 mutations in the SEPN1 gene. Mutations c.611dupA, c.713dupA, and c.583G>A were described previously [24, 25].

C.611dupA is a frameshift mutation that produces a shortened dysfunctional protein. This mutation was homozygous in patient 5, which is consistent with an autosomal recessive pattern of inheritance.

Another frameshift mutation c.713dupA was detected in a compound heterozygous patient 4. It is described as a cause of rigid spine muscular dystrophy (OMIM entry #602771) in homo- and heterozygous French patients [24]. Genetic variant c.583G>A carried by patient 4 was characterized by the prediction software as likely pathogenic; genetic databases refer to it as benign. Therefore, its role in the disease still needs to be elucidated.

Mutation c.485C>A is a previously unknown mutation that was heterozygous in patient 6. This means that multicore myopathy diagnosed in this patient cannot be confirmed by the genetic test, although morphological and clinical findings suggest otherwise. At the same time, we cannot rule out SEPN1- associated multicore myopathy because mutations in the second allele of the gene might have been overlooked due to the technical limitations of massive parallel sequencing.

Sequencing revealed the absence of RYR1 and SEPN1 mutations in 2 patients (patients 7 and 8) who had been diagnosed with myopathy/myocystrophy before the genetic test and had a morphological phenotype of central core disease.

Table 1. Symptoms of the patients with morphological signs of core myopathies revealed by histological analysis

<table>
<thead>
<tr>
<th>Symptoms / Patient</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight and height at birth (g/cm)</td>
<td>3310/50</td>
<td>3540/55</td>
<td>2480/48</td>
<td>2800/49</td>
<td>3780/53</td>
<td>3060/50</td>
<td>3085/50</td>
<td>2859/50</td>
</tr>
<tr>
<td>Congenital hip dislocation/dysplasia</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+ (dislocation)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Delayed motor development</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Muscle strength</td>
<td>4 points</td>
<td>4 points</td>
<td>4 points</td>
<td>2–3 points</td>
<td>4 points</td>
<td>2–3 points</td>
<td>1–2 points</td>
<td>3–4 points</td>
</tr>
<tr>
<td>Gait</td>
<td>Myopathic</td>
<td>Myopathic</td>
<td>Myopathic</td>
<td>Myopathic</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Spinal deformities</td>
<td>Thoracic hyperkyphosis</td>
<td>Thoracic hyperkyphosis</td>
<td>Curvature of the spine</td>
<td>Thoracic and lumbar spine scoliosis</td>
<td>Rigid spine</td>
<td>Kyphoscoliosis</td>
<td>Scoliosis</td>
<td>Scoliosis</td>
</tr>
<tr>
<td>Joint contractures</td>
<td>Ankle joint</td>
<td>–</td>
<td>–</td>
<td>Hip, knee and ankle joints</td>
<td>Ankle joint</td>
<td>Hip, knee and ankle joint</td>
<td>Hip, knee, ankle, elbow and wrist joints</td>
<td>Hip, knee and ankle joint</td>
</tr>
<tr>
<td>Reduced reflexes in upper and lower limbs</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Breathing problems</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Reduced intellectual capacity</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Additional symptoms</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Severe malnutrition</td>
<td>–</td>
<td>Piosis of the upper eyelid, chronic hypoventilation syndrome</td>
<td>Weak facial muscles, occipital epilepsy</td>
<td>–</td>
</tr>
<tr>
<td>CPK levels, un/L (normal range of 15–190)</td>
<td>78</td>
<td>66</td>
<td>79</td>
<td>188</td>
<td>284</td>
<td>174</td>
<td>290</td>
<td>194</td>
</tr>
</tbody>
</table>

Note: 1 — boy, 4 years; 2 — girl, 3 years; 3 — girl, 5 years; 4 — girl, 8 years; 5 — boy, 7 years; 6 — girl, 11 years; 7 — girl, 14 years; 8 — girl, 7 years.
Table 2. A list of mutations detected in the patients with morphological signs of core myopathy

<table>
<thead>
<tr>
<th>Patient</th>
<th>Diagnosis before the genetic test</th>
<th>Gene</th>
<th>Exon N°</th>
<th>Transcript N°</th>
<th>Nucleotide</th>
<th>Amino acid substitution</th>
<th>Genotype</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Congenital structural myopathy. Central core disease</td>
<td>RYR1, 19q13.2</td>
<td>86</td>
<td>NM_000540.2</td>
<td>c.11798A&gt;G</td>
<td>p.Tyr3933Cys</td>
<td>Heterozygous</td>
<td>[21]</td>
</tr>
<tr>
<td>2</td>
<td>Congenital myopathy</td>
<td>RYR1, 19q13.2</td>
<td>100</td>
<td>NM_000540.2</td>
<td>c.14387A&gt;G</td>
<td>p.Tyr4796Cys</td>
<td>Heterozygous</td>
<td>[22]</td>
</tr>
<tr>
<td>3</td>
<td>Congenital structural myopathy</td>
<td>RYR1, 19q13.2</td>
<td>101</td>
<td>NM_000540.2</td>
<td>c.14581C&gt;T</td>
<td>p.Arg4861Cys</td>
<td>Heterozygous</td>
<td>[23]</td>
</tr>
<tr>
<td>4</td>
<td>Congenital myopathy</td>
<td>SEPN1, 1p36.11</td>
<td>47</td>
<td>NM_000540.2</td>
<td>c.7561G&gt;A</td>
<td>p.Tyr2521Met</td>
<td>Heterozygous</td>
<td>Not described</td>
</tr>
<tr>
<td>5</td>
<td>Congenital myopathy</td>
<td>SEPN1, 1p36.11</td>
<td>5</td>
<td>NM_002451.2</td>
<td>c.713dupA</td>
<td>p.Asn238fs</td>
<td>Heterozygous</td>
<td>[24]</td>
</tr>
<tr>
<td>6</td>
<td>Congenital myopathy</td>
<td>SEPN1, 1p36.11</td>
<td>5</td>
<td>NM_002451.2</td>
<td>c.583G&gt;A</td>
<td>p.Asn195Thr</td>
<td>Heterozygous</td>
<td>[25]</td>
</tr>
<tr>
<td>7</td>
<td>Congenital structural myopathy</td>
<td>LAMA2, 6q22.33</td>
<td>23</td>
<td>NM_000426.3</td>
<td>c.3406T&gt;C</td>
<td>p.Cys1136Arg</td>
<td>Heterozygous</td>
<td>Not described</td>
</tr>
<tr>
<td>8</td>
<td>Congenital muscular dystrophy</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Unknown</td>
<td>–</td>
</tr>
</tbody>
</table>

Note: 1 — boy, 4 years; 2 — girl, 3 years; 3 — girl, 5 years; 4 — girl, 8 years; 5 — boy, 7 years; 6 — girl, 11 years; 7 — girl, 14 years; 8 — girl, 7 years; * — a stop codon.

Patient 7 had 2 presumably compound heterozygous mutations in the LAMA2 gene (14.28% of total detected mutations). Mutant LAMA2 variants are associated with type 23 limb-girdle muscular dystrophy (OMIM entry #618138) and congenital merosin-deficient muscular dystrophy (OMIM entry #607855) that follow a dominant recessive pattern of inheritance.

The LAMA2 mutation c.7147C>T (p.Arg2383*) detected in patient 7 results in the synthesis of a shortened dysfunctional protein. Its homozygous variant was previously described in a 4-year-old girl [28] who had typical symptoms of type A merosin-deficient muscular dystrophy, including congenital hypotonia, muscle weakness, elevated CPK of 1,556 IU/L and white matter abnormalities seen on MRI. Besides, patients affected with this disorder can have seizures and structural brain abnormalities. In patients with congenital laminin alpha 2 deficient muscular dystrophy, severity of the clinical symptoms varies, but the causes underlying this phenomenon are not fully understood and might be associated with RNA missplicing [26].

The second detected mutation in the LAMA2 gene is a nonsynonymous substitution c.3406T>C (p.Cys1136Arg) that was not described previously. Nonsynonymous substitutions can result in the formation of alternative splice sites, synthesis of new protein isoforms and conformational changes to the protein structure that affect its function. Therefore, the role of the detected mutation in the development of the disease needs to be elucidated.

The symptoms observed in patient 7 were different from those described above. The differential diagnosis included Werdnig-Hoffmann disease and congenital structural myopathy. Epilepsy was benign and was not considered a symptom. Histological findings suggested central core disease. However, the clinical, morphological and genetic data collected from the patient should not be regarded as controversial. The mechanism underlying the formation of cores in muscle fibers and the time it takes remain understudied [2]. Formation of cores might be the result of disrupted mitochondrial activity. The study of muscle tissue biopsy samples obtained from patients with different forms of congenital myopathy/myodystrophy at different stages of the disease will broaden our knowledge of the interactions and the order of involvement of proteins and muscle tissue components into the pathological process.

No clinically relevant genetic variants associated with neuromuscular disorders were detected in patient 8. However, this might have been due to the technical limitations of massive parallel sequencing.

The absence of mutations in the RYR1 gene in 2 patients (7 and 8) with a preliminary diagnosis of congenital myopathy/myodystrophy and a morphological phenotype of central core disease confirms the need for extensive molecular genetic testing in such patients. At the same time, in the presence of additional clinical symptoms rarely seen in a particular condition (in our case, patient 7 had seizures) the probability of detecting other molecular-genetic abnormalities increases. This also speaks for the necessity of research into the mechanisms underlying congenital myopathies and myodystrophy and their morphological manifestations.

We have also discovered correlations between CPK activity and the detected mutations. As a rule, CPK levels suggest the location of lesions in patients with neuromuscular disorders, their acuteness and duration. RYR1 mutations were present only in the patients with normal CPK levels. The highest CPK was observed in the patient with a homozygous SEPN1 mutation and also in the patient who carried mutations in the LAMA2 gene associated with the most severe form of congenital myopathy. Perhaps, CPK can be used to measure pathogenicity of a molecular-genetic abnormality (the presence/absence of a protein or the loss of its function) that leads to the disease and causes secondary myodystrophy.

CONCLUSIONS

Our findings confirm that myopathies characterized by the presence of cores in muscle fibers are genetically heterogeneous. Mutations in the RYR1 and SEPN1 genes are the major genetic cause of core myopathies in Russian patients, which is consistent with the findings of our foreign colleagues. The majority (75%, 6 of 8 patients) of RYR1 and SEPN1 mutant variants carried by our patients were described previously. Two previously unknown mutations need to be studied further in order to elucidate their clinical relevance. Our work shows that histological findings cannot be used as the only criterion for the differential diagnosis of congenital myopathies. Morphological phenotypes typical for core myopathies can also be seen in other congenital myopathies.
or myodystrophy. This means that clinical, morphological and genetic correlations should be studied in-depth to understand the mechanisms underlying the development of the disease and to come up with effective therapies in the case of complications. The absence of mutations in the genes implicated in congenital myopathies in patients with clinical symptoms and morphological signs of core myopathies requires further investigation.

References


