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 heterogenous 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 children were included in the study: 2 boys and 6 girls aged 3 to 14 years. Their biopsy material was analyzed by light and electron microscopy. Sequencing was performed on HiSeq2500. Mutations were detected in 7 (87.5%) of 8 participants. Six children had 8 mutations in the 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 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

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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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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 массового 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 мерозин-негативную мышечную дистрофию. Доли пациентов с выявленными мутациями в гене *RYR1* и мутациями в гене *SEPN1* одинаковы и составили 42,86% среди пациентов с мутациями. Из 10 мутаций, выявленных у обследованных пациентов, 3 мутации описаны впервые: в гене *RYR1* — c.7561G>A; в гене *SEPN1* — c.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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Congenital myopathies constitute a clinically and genetically heterogenous 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 pathology 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.

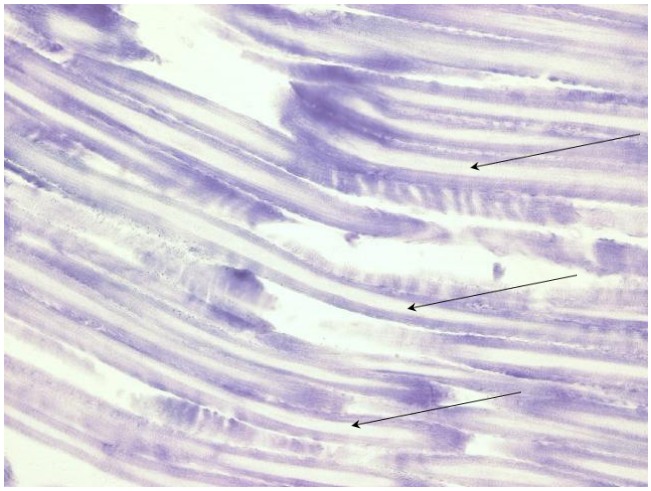

Fig. 1. Skeletal muscle tissue of a 21-year-old patient with congenital central core myopathy. Histochemical analysis of succinate dehydrogenase activity in frozen sections (×200; the method was proposed by Sukhorukov VS) using the nitro BT method by Nachlass et al. (1957). The total absence of enzyme activity is observed in almost all longitudinal sections of the examined muscle fibers (*the arrows*)

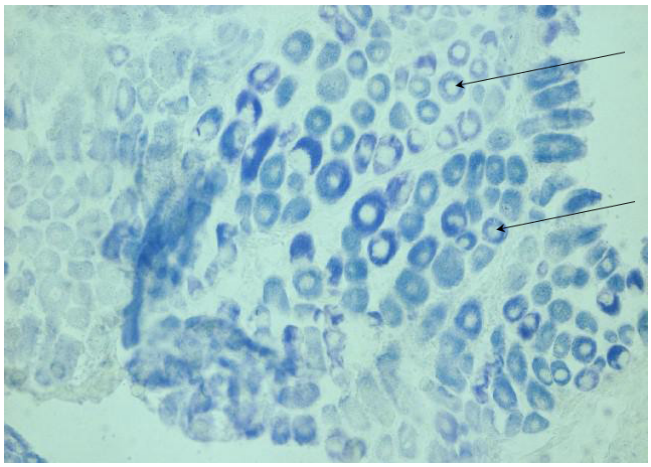

Fig. 2. Skeletal muscle tissue of a 1-year-old patient with congenital central core myopathy Histochemical analysis of succinate dehydrogenase activity in frozen sections (×200; the method was proposed by Sukhorukov VS) using the nitro BT method by Nachlass et al. (1957). The total absence of enzyme activity is observed in almost all cross-sections of the examined muscle fibers (*the arrows*)

Histologically, multicore 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 multiminicore myopathy, the cores are quite massive [9, 11]. In *SEPN1*-associated myopathy, minicores are abundantly present in the muscle tissue [9, 11].

Multiminicore 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 (pH 7.4) buffered formalin following the manufacturer's protocol and then slicing it on the microtome.

2. Hematoxylin-eosin staining of both frozen and paraffinized sections was performed according to the standard protocol. Succinate dehydrogenase activity in the frozen sections was measured using the standard nitro blue tetrazolium-based technique proposed by Nachlass in 1957 [16].

3. Prior to electron microscopy, the samples less than 1 mm^3 in size were prefixed in a 2.5% solution of glutaraldehyde in a phosphate buffer (pH 7.4) at 0 °C. After being washed in a phosphate buffer (pH 7.4), the samples were fixed in 1% osmium tetroxide at 2–4 °C for 1 h. Then, the samples were dehydrated in a series of ascending ethanol concentrations, washed in acetone 3 times and embedded in Epon resin using a technique proposed by Luft [17]. The slices were prepared on the REICHERT Nr. 321850/E ultratome (Reichert tech.; Austria) and stained with lead citrate and sodium uranyl acetate as proposed by Reynolds in [18]. The sections were examined under a transmission electron microscope JEOL JEM-100B (Jeol LTD; Japan).

Exome sequencing was performed using the HiSeq2500 platform (Illumina; USA) according to the manufacturer's protocol.

1. Amplification products were labeled using the BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific; USA) according to the manufacturer's protocol. Sanger sequencing was carried out on an ABI PRISM 3500 Genetic Analyzer (Applied Biosystems; USA) according to the manufacturer's protocol.

2. Sequencing data was processed in Cutadapt ver. 1.14 (Cutadapt; USA), BWA ver. 1.14 (Illumina; USA), FastQC ver. 0.11.5 (Illumina; USA), GATK HaplotypeCaller ver. 3.7 (GATK HaplotypeCaller; USA), and snpEff ver. 4.3p (SnpEff; USA). Pathogenicity and conservation of mutations were assessed based on the data extracted from dbNSFP, Clinvar, OMIM, and HGMD databases. SIFT 1.03 (SIFT; USA) and Polyphen2 ver. 2.2.2 (Polyphen2; USA) utilities were used to predict potential pathogenicity of the detected mutations. Information about the frequency of the mutations was obtained from the 1000Genomes project, ExAC and other databases. The annotations and pathogenicity of the detected mutations were predicted as proposed by the Standards and Guidelines for the interpretation of sequence variants developed by ACMG, AMP and CAP [19].

3. Clinical interpretation of sequencing data was aided by OMIM (https://omim.org/), Varsome (https://varsome.com/), ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), The Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac/index.php), and some other genetic databases according to the guidelines for MPS data interpretation [20].

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

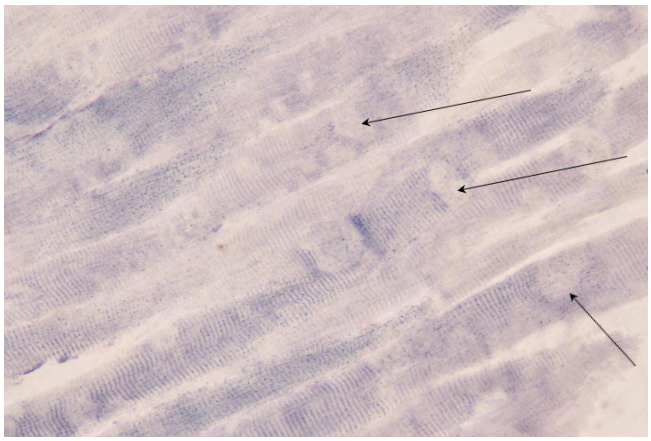

Fig. 3. Skeletal muscle tissue of a 3.5-year-old patient with congenital multiminicore myopathy Histochemical analysis of succinate dehydrogenase activity in frozen sections (×200; the method was proposed by Sukhorukov VS) using the nitro BT method by Nachlass et al. (1957). Cores are abundant in almost all longitudinal sections of the examined muscle fibers (*the arrows*)

phosphokinase (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/myodystrophy 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

Symptoms / Patient	1	2	3	4	5	6	7	8
Weight and height at birth (g/cm)	3310/50	3540/55	2480/48	2800/49	3780/53	3060/50	2859/50	3500/54
Congenital hip dislocation/dysplasia	–	–	+ (dislocation)	–	–	–	+ (dysplasia)	–
Delayed motor development	–	+	+	+	+	–	++	+
Muscle strength	4 points	4 points	4 points	2–3 points	4 points	2–3 points	1–2 points	3–4 points
Gait	Myopathic	Cannot walk at the moment	Myopathic	Myopathic	Myopathic	Has not walked since 9 years of age	Was never able to walk	Myopathic
Spinal deformities	Thoracic hyperkyphosis	Thoracic hyperkyphosis	Curvature of the spine	Thoracic and lumbar spine scoliosis	Rigid spine	Kyphoscoliosis	Scoliosis	Scoliosis
Joint contractures	Ankle joint	–	–	Hip, knee and ankle joints	Ankle joint	Hip, knee and ankle joint	Hip, knee, ankle, elbow and wrist joints	Hip, knee and ankle joint
Reduced reflexes in upper and lower limbs	+	+	+	++	+	++	++	++
Breathing problems	+	+–	N/A	+++	+	+	N/A	+
Reduced intellectual capacity	–	+	+ –	–	–	–	–	–
Additional symptoms	–	–	–	Severe malnutrition	–	Ptosis of the upper eyelid, chronic hypoventilation syndrome	Weak facial muscles, occipital epilepsy	–
CPK levels, un/L (normal range of 15–190)	78	66	79	188	284	174	290	194

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

Patient	Diagnosis before the genetic test	Gene	Detected mutations and genotypes					Reference
			Exon №	Transcript №	Nucleotide	Amino acid substitution	Genotype	
1	Congenital structural myopathy. Central core disease	*RYR1*, 19q13.2	86	NM_000540.2	c.11798A>G	p.Tyr3933Cys	Heterozygous	[21]
2	Congenital myopathy	*RYR1*, 19q13.2	100	NM_000540.2	c.14387A>G	p.Tyr4796Cys	Heterozygous	[22]
3	Congenital structural myopathy	*RYR1*, 19q13.2	101	NM_000540.2	c.14581C>T	p.Arg4861Cys	Heterozygous	[23]
			47	NM_000540.2	c.7561G>A	p.Val2521Met	Heterozygous	Not described
4	Congenital myopathy	*SEPN1*, 1p36.11	5	NM_020451.2	c.713dupA	p.Asn238fs	Heterozygous	[24]
			5	NM_020451.2	c.583G>A	p.Ala195Thr	Heterozygous	[25]
5	Congenital myopathy	*SEPN1*, 1p36.11	4	NM_206926.1	c.611dupA	p.Asn204fs	Homozygous	[24]
6	Congenital myopathy	*SEPN1*, 1p36.11	4	NM_206926.1	c.485C>A	p.Ser162*	Heterozygous	Not described
7	Congenital structural myopathy	*LAMA2*, 6q22.33	50	NM_000426.3	c.7147C>T	p.Arg2383*	Heterozygous	[26, 28]
			23	NM_000426.3	c.3406T>C	p.Cys1136Arg	Heterozygous	Not described
8	Congenital muscular dystrophy	–	–	–	Undetected	Undetected	Unknown	–

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 afflicted 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 heterogenous. 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.

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