UNIVERSIDADE ESTADUAL DE CAMPINAS

FACULDADE DE CIÊNCIAS MÉDICAS

TATIANA DA SILVA ROSA

MIOPATIAS COM CENTRALIZAÇÃO NUCLEAR

CENTRONUCLEAR MYOPATHIES

CAMPINAS

2018

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Tese apresentada à Faculdade de Ciências Médicas da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do título de Doutora em Ciências Médicas, área de concentração Ciências Biomédicas.

Thesis presented to the Faculty of Medical Sciences, University of Campinas as part of the requirements for obtaining the title PhD in Medical Sciences, area of concentration Biomedical Sciences.

ORIENTADOR: ANAMARLI NUCCI

ESTE EXEMPLAR CORRESPONDE À VERSÃO FINAL DA TESE DEFENDIDA PELA ALUNA TATIANA DA SILVA ROSA, E ORIENTADO PELA PROFª. DRª. ANAMARLI NUCCI.

CAMPINAS

2018

Agência(s) de fomento e nº(s) de processo(s): CAPES, 01-P-3368/2017

Ficha catalográfica
Universidade Estadual de Campinas
Biblioteca da Faculdade de Ciências Médicas
Maristella Soares dos Santos - CRB 8/8402

R71m

Rosa, Tatiana da Silva, 1984-
Miopatias com centralização nuclear / Tatiana da Silva Rosa. – Campinas, SP : [s.n.], 2018.

Orientador: Anamarli Nucci.
Tese (doutorado) – Universidade Estadual de Campinas, Faculdade de Ciências Médicas.

1. Miopatias congênitas estruturais. 2. Ressonância magnética. 3. Escala Medida da Função Motora. I. Nucci, Anamarli, 1947-. II. Universidade Estadual de Campinas. Faculdade de Ciências Médicas. III. Título.

Informações para Biblioteca Digital

Título em outro idioma: Centronuclear myopathies
Palavras-chave em inglês:
Myopathies, Structural, Congenital
Magnetic resonance imaging
Motor function measure scale
Área de concentração: Neurologia
Titulação: Doutora em Ciências Médicas
Banca examinadora:
Anamarli Nucci [Orientador]
Umbertina Conti Reed
Helga Cristina Almeida Silva
Fabio Rogério
Sérgio San Juan Dertkigil
Data de defesa: 21-02-2018
Programa de Pós-Graduação: Ciências Médicas

BANCA EXAMINADORA DA DEFESA DE DOUTORADO

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A ata de defesa com as respectivas assinaturas dos membros da banca examinadora encontra-se no processo de vida acadêmica do aluno.

Data: 21/02/2018

Dedico esta tese a minha avó Natalia *(in memoriam)* ***que me mostrou que gastar a vida acreditando no melhor das pessoas e construindo um mundo com justiça e igualdade, ajudando as pessoas a se tornarem o melhor que elas podem ser, é a melhor forma de marcar nossa curta passagem pelo mundo****.*

AGRADECIMENTOS

Agradeço à minha professora-orientadora Anamarli Nucci, que teve paciência, que me ajudou a concluir este trabalho e pelo acolhimento fraterno. Muito obrigada!!

À minha família, por sua capacidade de acreditar e investir em mim. Mãe, seu cuidado e dedicação são que me dão, em alguns momentos, a esperança para seguir. Minhas irmãs (Daniela, Tallita, Hyngrid, Victoria e Thais) e sobrinhos (Ana Luiza, João, Maria, Sophia e Alice) que tanto amo, muito obrigada por vocês existirem. Aos meus tios e tias, em especial ao meu tio Valdemir.

Aos funcionários e residentes do Departamento de Neurologia e Ambulatório de Neurologia, por partilhar as salas e por todo auxílio na busca dos pacientes.

À Marcinha da Comissão de Pós-graduação por todas as orientações desde os anos mestrado.

À professora Elisa do Departamento de Radiologia pelo auxílio com as imagens dos nossos casos.

Ao professor Luciano do Departamento de Patologia pelos dados de anatomia patológica dos nossos casos.

Ao professor Marcondes e toda equipe da Neuromuscular.

À minha amiga de ambulatório e, de vida de tantos anos, Cristina Iwabe. Muito obrigada pelo apoio e dedicação.

Pela participação e disponibilidade, agradeço aos pacientes e voluntários envolvidos nesta tese.

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) pela Bolsa de Formação de Pesquisador de Doutorado.

Aos amigos professores da UNIFAJ, pela alegre e leve concivência.]

À minha amiga e vizinha Milene e à minha sobrinha de coração Sophie, foram 4 agitados anos. Muito obrigada.

À minha amiga parceira Cibele, muito obrigada por me aturar e aturar as minhas neuras, por todas as leituras e revisões nos textos. Muito obrigada pela convivência e pelo ouvido amigo em tantos momentos.

Aos meus grandes amigos de perto (Lívia, Rafaela, Layane, Manoela, Juliana, Regiane, Marina, Patrícia e Daniel) e de longe (Cris, Rafael, Lhais, Cido, Sol, Nieves e Lucas) e tantos outros que de alguma forma estiveram e estão próximas a mim, fazendo esta vida valer cada vez mais a pena.

RESUMO

As miopatias centronuclear (MCN) e miotubular (MMT) são doenças congênitas, estruturais e raras que apresentam núcleo central nas fibras musculares. Apresenta-se a variabilidade fenotípica e genética dessas miopatias em revisão bibliográfica no Capítulo 1. **Objetivos:** conhecer a funcionalidade motora e padrões de ressonância magnética muscular (RMm) em coorte de pacientes com MCN e MMT. Descrever casos incomuns da coorte, seja por aspectos genéticos ou anatomopatológicos. **Métodos:** usou-se a escala Medida da Função Motora, em português (MFM-P); protocolo de RMm, segundo DeCauwer et al e graduação da intensidade da infiltração gordurosa de zero (músculo normal) a 4 (músculo lipo-substituído), segundo Mercuri et al. As biopsias musculares seguiram técnicas de acordo com Dubowitz e o exame de genética molecular usou seqüenciamento de nova geração (NGS) para painel de genes. **Resultados:**No Capítulo 2, descreveu-se paciente com nova mutação causal no gene da miotubularina e um outro com a síndrome de genes contíguos (*MTM1/MAMLD1*), no qual a informação sobre RMm é inédita para casos pediátricos.No capítulo 3, descreveu-seum caso com características anatomopatológicas sugestivas de possível mutação no gene *BIN1;*porém com genética molecular resultando em mutação*DNM2*, portanto, expandindo o fenótipo morfológico da MCN-*DMN2*.A RMm mostrou padrão compatível com o dado genético. No capítulo 4, dez pacientes foram estudados com MFM-P e RMm. O escore médio total MFM-P foi de 64,26% na primeira avaliação (normal 100%). A RMm graduou a gravidade da doença, expressa por infiltração gordurosa muscular, em cada caso. O exame de RMm, em conseqüência do protocolo utilizado, reforçou os dados de literatura em relação aos músculos da pelves, coxas e pernas e acrescentou informações sobre os músculos paravertebrais cervicais, escapulares e de braços, com destaque para o comprometimento dos paravertebrais, do deltóide (anterior e médio) e cabeça curta do bíceps braquial.**Conclusão:** a escala MFM-P e a RMm são métodos não invasivos capazes de mostrar a gravidade da MCN e a genética molecular definiu quatro casos da coorte.

Palavras chave: Miopatias Congênitas Estruturais, Ressonância magnética, Escala Medida da Função Motora, versão em português.

ABSTRACT

Centronuclear (CNM) and myotubular myopathy (MTM) are congenital, structural and rare diseases that have a central nucleus in muscle fibers. The phenotype and genetic variability of these myopathies is reviewed in Chapter 1. **Objectives:** To know motor function severity and muscle magnetic resonance (mMR) patterns in a cohort of CNM and MTM patients. To describe cases having genetic or pathological uncommon aspects. **Methods:** We used Motor Function Measurement scale in Portuguese (MFM-P) and mMR images according to DeCauwer et al protocol. The degree of fat infiltration intensity was graded zero (normal muscle) to 4 (total fat infiltration), according to Mercuri et al. Muscle biopsies followed techniques described by Dubowitz and the molecular genetics examination used new generation sequencing (NGS) in a panel of gene. **Results:** In Chapter 2 we described a patient with de novo mutation in the myotubularin gene and another with contiguous gene syndrome (*MTM1/ MAMLD1*), in which mMR is firstly described for pediatric patient. In Chapter 3, a case of *DNM2* mutation is reported because anatomopathological characteristics suggestive of *BIN1*-CNM, thus expanding the morphological phenotype of CNM-*DMN2*. Muscle MR was in accordance with genetic data. In Chapter 4, ten patients were studied with MFM-P and mMR. The mean total MFM-P score was 64.26% in the first evaluation (normal 100%). Muscle MR graded the severity of the disease, expressed by muscular fatty infiltration, in each case. The MRI examination, as a consequence of the protocol used, reinforced the literature data regarding the muscles of the pelvis, thighs and legs and added information about the paravertebral cervical, scapular and arm muscles. Paravertebral muscles, deltoid (anterior and middle) and short head of the biceps brachii were more affected. **Conclusion:** MFM-P scale and mMR are noninvasive tools able to show the severity of CNM and the molecular genetics defined four cases of the cohort.

Keywords: Myopathies, Structural, Congenital, Magnetic Resonance Imaging, Motor function measure scale.

LISTA DE ILUSTRAÇÕES

LISTA DE ABREVIATURAS E SIGLAS

AD	Herança autossômica dominante
AR	Herança autossômica recessiva
BIN1	Gene da Anfifisina 2
CK	Creatinaquinase
D1	Dimensão 1 da MFM-P
D2	Dimensão 2 da MFM-P
D3	Dimensão 3 da MFM-P
DNM2	Gene da Dinamina 2
DP	Desvio padrão
FCM	Faculdade de Ciências Médicas
LX	Herança ligada ao cromossomo X
HC	Hospital de Clínicas
H&E	Hematoxilina e eosina
IMC	Índice de Massa Corpórea
MFM	Medida da Função Motora
MFM 32-P	Medida da Função Motora – versão em português
MMT	Miopatia miotubular
MTT-LX	Miopatia miotubular ligada ao X
MCN	Miopatia centronuclear
MCN-AD	Miopatia centronuclear autossômica dominante
MCN-AR	Miopatia centronuclear autossômica recessiva
MTM1	Gene da Miotubularina
RYR1	Gene da Rianodina 1
SPEG	Gene da Proteina quinase preferenciamente expressa em músculo estriado
TTN	Gene da Titina
UNICAMP	Universidade Estadual de Campinas

SUMÁRIO

1.0 INTRODUÇÃO GERAL

As miopatias com centralização nuclear são miopatias congênitas e estruturais raras, heterogêneas à clínica e quanto aos defeitos genéticos [1-3]. São conceituadas, fundamental e consensualmente, pela presença de núcleo central em fibras musculares, observado à biópsia muscular [1-2].

A incidência das miopatias congênitas é cerca de 0,06 / 1000 nascidos vivos ou um décimo de todos os casos de doenças neuromusculares [4]. Estudos regionais, como os realizados na Irlanda do Norte [5] e Suécia ocidental [6] sugeriram uma prevalência de 3,5 – 5,0 / 100.000 da população pediátrica nos anos de 1990 e 2000, respectivamente [5,6]. No Reino Unido, 7 entre 56 biópsias de pacientes com miopatias congênitas foram diagnosticas como centronuclear/miotubular (13%), num período de 5 anos, segundo Catteruccia *et al* [7]. Laport *et al* [8] estimaram a incidência da miopatia miotubular ligada ao X (MMT-LX), na França, em 2/100.000 nascimentos masculinos ao ano, estimativa esta que teve por base os casos confirmados pela genética molecular. Segundo Jungbluth *et al* [3] a prevalência da miopatias centronuclear (MCN) ocorre com menor frequência do que as miopatias dos focos centrais, dos multiminifocos e da miopatia nemalínica.

No Brasil, o primeiro caso descrito de miopatia miotubular (MMT) foi em 1977 [9], seguido de outros relatos de casos isolados [10-13]. Uma série de casos nacionais foi apresentada por Zanoteli *et al* em 1998 [14-15]. Em 2013 a autora presente trabalho, em dissertação de mestrado, apresentou uma serie de 13 casos [16] e Abath Neto *el al* [17], em doutorado, 18 casos. O autor descreveu 10% de MCN em duas instituições publicas referencias em doenças neuromusculares: 6 casos do tipo MCN ligado ao X [18] e dois do subgrupo MCN com mutação na dinamina, casos esporádicos [19]. Doze casos brasileiros de MCN com mutação

RYR1 fizeram parte de publicação internacional [20].

A maior facilidade de diagnóstico molecular nas miopatias congênitas e naquelas com centralização nuclear, tem permitido melhor caracterização das mesmas. A analise de DNA tem como vantagem utilizar-se de amostras de sangue periférico e ser exame minimamente invasivo. Na linha de exames não invasivos que acrescentam importante conhecimento sobre as miopatias em geral esta a RMm [21] e em especial na MCN [7].

Colaborando na avaliação não invasiva dos pacientes com miopatias tem sido descrito o estudo funcional com o uso de escalas, como por exemplo a escala Medida da Função Motora [22,23,24]. No sentido de avaliação não invasiva de pacientes MCN focamos o presente trabalho.

2.0 CAPÍTULO 1 - REVISÃO LITERATURA

CENTRONUCLEAR MYOPATHIES: REVIEW

Tatiana Silva Rosa, José Darlan Pinheiro Domingues, Marcondes Cavalcante França Jr, Anamarli Nucci

Centronuclear myopathies (CNM) are rare congenital and structural muscle disorders characterized by clinical and genetic heterogeneity (1,2). The main biopsy feature is a large central nucleus in a variable proportion of muscle fibers and some other peculiarities that aid in the diagnosis of CNM subtypes (1).

In the years 1960-70, the terms myotubular myopathy (MTM) and CNM were used to designed congenital myopathies with similar pathological expressions but distinct physiopathogenic hypothesis or they were also used as a synonym (3-6). After the years 1990, a consensus was reached designating MTM as the X-linked myopathy (XLMTM) and CNM for cases with autosomal inheritance (7). Up to date, seven different genes are implicated in the etiology of CNM.

The aim of the study is to review the clinical, genetic, laboratory and pathophysiology of CNM subtypes.

Historical notes

Spiro, Shy and Gonatas (3), (1966), were responsible for the first description of a congenital muscle disorder that had a large central nucleus within small muscle fibers resembling myotubes and the authors coined it MTM. Similar anatomopathological aspects were seen in cases from Sher et al (4) (1967) but named CNM. Wijngaarden et al (5) (1969) reported a large family from Holland with XLMTM. Vital et al ref (6) (1970) published an adult-onset case of CNM. MacLeod et al (7) (1972) described a family with autosomal dominant inheritance (CNM-AD). Darnfors et al (8) and Thomas et al (9) showed the linkage of MTM with the locus Xq28 and Laporte et al (10) (1996) identified the gene *MTM1* as the cause of XLMTM. Bitoun et al (11) (2005) discovered the first causative gene for CNM-AD, the *DNM2* gene and, Jungbluth et al (12) (2007) CMN-AD caused by *RYR1* gene. Nicot et al (13) (2007) were able to be showed that mutations in

the gene *BIN1* were cause of autosomal recessive CNM (CNM-AR). Ceyhan-Birsoy et al (14) (2013) observed that CNM-AR may also be caused by *TTN* gene mutations. Böhnm et al. (15) (2014) showed that mutations in the gene *BIN1* are also cause of CNM-AD. Agrawal et al (16) reported the last know gene associated with CNM-AR, that is, *SPEG* complex locus. All the above mentioned exemplify the diversity of genetic inheritance and etiology in CNM (Table.1)

Table 1. Genes involved in centronuclear myopathies.

Gene	GENETIC PRODUCT	Locus	INHERITANCE	REFERENCE
MTM1 **(MIM*300415)** **(OMIM#310400)**	Miotubularin	*Xq28*	XR	Laporte et al. (1996)
DNM2 **MIM*602378)** **(OMIM#160150)**	Dinamin 2	*19p13.2*	AD	Bitoun et al. (2005)
RYR1* **(MIM*180901)** **(OMIM#117000)**	skeletal muscle ryanodine receptor	*19q13.2*	AD	Jungbluth et al. (2007)
BIN1 **(MIM*601248)** **(OMIN#255200)**	Amphiphysin-2	*2q14.3*	AR	Nicot et al. (2007)
BIN1 **MIM*** **(OMIM#255200)**	Amphiphysin-2		AD	Böhm et al. (2014)
TTN **(MIM*188840)**	Titin	*2q31.2*	AR	Ceyhan-Birsoy et al. (2013)
SPEG complex **(MIM*615950)** **OMIM#615959**	SPEG	*2q35*	AR	Agrawal et al. (2014)

Legend: XL: X-Linked; AD: autossomal dominant; AR: autossomal recessive

Epidemiology of CNM

There is a lack in the epidemiological knowledge of congenital myopathies, although authors (2) say CNM has low frequency in relation to central core, multiminicore and nemaline myopathies. In France, Laporte esteemed 2/100.000 male births per year as having XLMTM, based in genetically defined cases (2).

In Brazil, the first case of MTM appeared in 1977 (17), others isolated cases were published in 1981 (18) and 1992 (19). In 1998, ten cases of CNM were published by Zanoteli et al. (20). Recently Abath Neto et al (21) described 18 muscle biopsies (22,8%) of CNM among congenital myopathies, issued from two public institutions that are national references in neuromuscular diseases, in the period of 2008-2013. Six XLMTM (21) and two sporadic cases of *DNM2*-CNM (22) were published. Twelve Brazilian cases of CNM with *RYR1* mutation were part of an international multicenter article (23).

Sex linked myotubular myopathy (XLMTM)

XLMTM (OMIM® #310400) is the X-linked type of CNM, in general, a severe congenital myopathy in males, caused by mutations in the *MTM1* gene (2,24). The classic type of this myopathy may have prenatal onset when polyhydramnios and reduced fetal movements may be detected. Affected male births with profound hypotonia and weakness usually associated with respiratory distress and feeding difficulties, obliging to intensive health care support. Most of these patients die in infancy or early childhood, some survive into later childhood, often with partial or total ventilation dependence, sometimes using nutritional support (25).

Additional phenotypic characteristics have been reported (2,26), as facial paresis, ophthalmoplegia/paresis, ogival palate, macrosomia, high stature, large cranial circumference and chryptorchidism.

Mild XLMTM cases are possible in males with neonatal onset, early infancy onset or beyond, although very rare (27). Intrafamilial variability was also described (28).

Female is generally an asymptomatic carrier of XLMTM or most rarely she is symptomatic, in both cases generally discovered by screening motivated by an affected boy in the family. Explanation for these symptomatic carriers is skewed

inactivation of X-chromosome. However, Savarese et al (29), highlighted the proximal weakness in girls to be caused by *MTM1* gene mutations, without XLMTM cases in their families. This is an alert to clinicians to extend genetic analysis in female with limb-girdle syndrome, when common panel for the syndrome was negative.

Interestingly, asymptomatic male was recently reported giving origin to an asymptomatic female carrier which had a child with severe XLMTM. The explanation to the inheritance via grandfather was early postzygotic mosaicism in this ancestry (30).

Clinicians must be attentive to comorbidities associated with XLMTM. The most life-threatening condition is peliosis hepatis due to potentially irreversible hemorrhage, especially in severe XLMTM (31). Peliosis is multiple cystic blood-filled spaces throughout the liver parenchyma with variable size so, superior abdominal images, like CT or MR, may favor the knowledge of vulnerable XLMTM-patients (32).

Ancillary exams like creatine kinase (CK) are generally normal or slightly elevated (2,33) and the gold standard for screening CNM is muscle biopsy (1,2,33). The exam shows predominance or uniformity of type 1 fiber; variable but generally high percentage of small type 1 fiber with great centralized nucleus (1,2,33,34).

Necklace fibers, is a peculiar finding firstly described in late-onset *MTM1* myopathy as a histological marker (33). These fibers may be seen even in hematoxylin and eosin stained muscle (H&E) as a basophilic ring underneath the sarcolemma and contouring all long the cell either in transverse or longitudinal plane sections. In some cells one or two nuclei may be aligned with the ring. Necklace fibers may also be seen in Gomori trichrome (GT) and periodic-acid Schiff (PAS) stains or cytochrome c oxidase (COX). It is also seen in nicotinamide adenosine dinucleotide–tetrazolium reductase (NADH-TR) histochemistry, but not in myosin ATPases (33), although type 1 and 2 muscle fibers may exhibit the ring. In electron microscopy, the necklace fibers exhibit normal aspects, except, for the correspondent basophilic ring that is always in equal distance of 3 millimicrons under the sarcolemma. It is distinguished by smaller and oblique myofibrils, increased number of mitochondria and sarcoplasmic reticulum profiles. Occasionally a normal nucleus is aligned with the ring (33). Necklace fibers may

be present not only in late-onset *MTM1* cases, but described in XLMTM (21).

Muscle images in *MTM1*-related myopathy are scarce. Only three late-onset cases had computed tomography (CT) and one of them had magnetic resonance (MR) after their thirties-years-old. The distinctive pattern of alterations was asymmetry of calves with soleus muscle most affected; gluteus, posterior thigh muscles and vastus intermedius showed major fat substitution. Rectus femoris, sartorius, gracilis and adductor longus were muscles relatively spared by the disease (33). It is important to note that muscle images have been an interesting tool in the approach of congenital myopathies in recent years (34).

***MTM1* gene and myotubularin protein**. The *MTM1* gene (MIM *300415), comprises 15 exons on Xp28 and codes for myotubularin protein. Up to 2012, over 200 mutations were identified in *MTM1,* like missense, nonsense, intronic, deletion, duplication and large rearrangement (35).

In neonatal intensive care unit, authors (36) indicated whole exome sequencing or next generation sequencing (NGS) for elucidated complex neuromuscular cases. This action defined some suspected CNM severe cases, with the advantage of preventing invasive or exhaustive laboratory examinations (34). However, when negative result is obtained in cases of high suspicion of XLMTM, remember to performer test: RT-PCR and or Western blotting may detect intronic mutation and MLPA or array-CGH testes for duplication/deletion of the gene (35). Analysis of mRNA by RT-PCR and sequencing may also unrevealing myotubularin decrease in muscle due to abnormal splicing (37).

Myotubularin has phosphatase and phosphoinositides functions that is involved in the endosomal-lysosomal pathway and is essential for muscle cell differentiation. It is also involved in regulation of mitochondrial morphology in muscle fibers by interaction with desmin (38).

Autosomal dominant dynamin 2 centronuclear myopathy (*DNM2*-CNM-AD)

The CNM-AD or CNM type 1 (OMIM #160150) was identified by Bitoun et al (11) in families with mutations in chromosome 19p13.2, locus coding the DNM2 protein. Latter, authors (39) identified de novo heterozygous mutations in the same locus in a case with neonatal-onset.

Mutations in dynamin 2 (*DNM2*) gene causing CNM-AD occurred in around 50% of patients, according to an Italian cohort (40).

CNM-AD may have a milder, neonatal to adult-onset muscle involvement (41). Facial weakness, bilateral ptosis and oftalmoparesis are seen in most patients, variably associated with distal muscle atrophy, finger and ankle contractures and pes cavus (42).

DNM2-related CNM are morphologically characterized by nuclear centralization more than internalization, radiating sarcoplasmic strands and type 1 fiber predominance and hypotrophy [1,11,40,41,43,44). It is to note that there are not marked regeneration (20).

Casar-Borota et al. (44) report a patient with *DNM2*-ate onset without symptoms that could be clearly related to CNM, associated morphological features of *DNM2*-CNM with the classical necklace fibers in the muscle biopsy. A novel pathogenic mutation in the proline-rich domain of *DNM2* supported the concept that the necklace fibers may occasionally be found in association with *DNM2* mutations. Other authors (40,45) described necklace fibers in *DNM2*-CNM, indicate possible common pathogenic mechanisms between *DNM2* and *MTM1* myopathies.

Several clinical features in *DNM2*-related CNM have been reported, ranging from mild adult-onset to severe evolution in infants-onset (11,40,42,46). Adult patients with *DNM2*-CNM usually present in adolescence or early adulthood with proximal or less often distal muscle weakness, combined with facial weakness or weakness in paraspinal muscles and neck flexors. Ptosis and ophthalmoplegia may also be early signs, however, not present in all patients. The muscle weakness is slowly progressive and may cause loss of ambulation in mid or late adulthood. A significant proportion of the patients develop restrictive respiratory difficulties later during the disease (46).

In patients with *DNM2*- CNM the electroneuromyography (EMG) show a myopathic pattern with discrete spontaneous activity. Neuropathic EMG features have previously been observed in some patients with DNM2-CNM, in addition to the predominant myopathic pattern, indicating possible overlap with the phenotype of Charcot Marie-Tooth disease (41,43,44).

Creatine kinase is normal or only slightly elevated, baring more common in nuclear centralization myopathies (1,2,47)

Muscle MRI shows a selective pattern of involvement in myopathies (48,49,50) with most severe involvement of the muscles of the distal lower leg and of the sartorius, adductor longus, biceps femoris and gluteus maximus muscles minor affected in cases of *DNM2*-CNM (40,41,43,49,50). This selective pattern is very characteristic for *DNM2*-CNM as the patterns of muscle involvement in other congenital myopathies caused by mutations in the *SEPN1*, *RYR1, NEB* or collagen VI encoding (*COL6A1, COL6A2, and COL6A3*) genes are different (51,52). Congenital myopathies related to *NEB* gene mutations have predominant anterior lower leg and mild anterior thigh compartment involvement which is opposed to the predominant posterior thigh and posterior lower leg involvement in *DNM2*-CNM (51). Patients with *RYR1* gene mutations have a more significant and earlier involvement of the anterior thigh compartment muscles and relative selective changes in the soleus muscle (52).

SEPN1 patients typically show an involvement of the sartorius and normal appearance of the gracilis muscles (52), while both muscles are relatively spared in patients with *DNM2*-CNM. Patients with Ullrich congenital muscular dystrophy or Bethlem myopathy which both are caused by (recessive or dominant) mutations in the three collagen VI encoding genes present with an early and peculiar involvement of the vastus lateralis muscle with relative sparing of the center of the muscle, a pattern not observed in *DNM2*-CNM.

***DNM2* gene and DMN2 protein.** *DNM2* gene (MIM*602378) is codified in chromosome 19p13.2, by 22 exons. A total of 18 different mutations of the *DNM2* gene were described involving exons 8 and 16, with 7 mutations for each of them. Mutation in exons 11, 14 and 15 is still occurred and one in the intrometric region (11). DNM2 protein is involved in endocytosis and membrane trafficking, actin assembly, and centrosome cohesion (11).

Autosomal recessive and autosomal dominant amphiphysin 2 related centronuclear myopathy (*BIN1*-CNM-AR and *BIN1*-CNM-AD)

Nicot et al (13) identified the first gene causative of AR-CNM (OMIM #255200). Homozygous *BIN1* (bridging integrator 1) mutations are associated with severe neonatal or childhood CNM with predominant proximal weakness (13) and is responsible for about 5% of AR-CNM (53).

One interesting case (54) differed from those of Nicot et al (13) due to mental retardation detected in early ages, ptosis, vertical ophthalmoparesis; facial, axial and proximal-distal weakness, that had progressive course. Fatigability was suspected in adulthood. A detailed case *BIN1*-CNM-AR was reported by Mejaddam et al (55).

BIN1 was also identified as responsible for AD-CNM (56). Nine patients were reported from five families. Clinical phenotype was characterized by absent facial weakness or major ocular problems, except mild ptosis (two cases) and vertical gaze limitations (two cases); predominant proximal lower limb deficit in all patients and in three, axial-distal muscle involvement were included. Cardiac signs or symptoms or significant respiratory dysfunction were not reported. Typical age-onset was over 22-year-old with progressive disability, although the majority of patients were ambulant at the time of publication.

Garibaldi et al (57) examined a family with *BIN1*-CNM-AD in which their members complained of myalgia. Recently, in 2017, Kouwenberg et al (58), reported a Dutch family with AD CNM due to a novel BIN1 mutation (c.53T>A (p.Val18Glu). The main features were mild proximal weakness with pronounced myalgia, exercise intolerance and muscle hypertrophy, with a childhood onset in the youngest generation, alongwith mild cognitive features. These two articles are important alert to clinicians to considered BIN1 mutations in patients with isolated exercise intolerance and/or myalgia, even in childhood.

Ancillary exams in patients with AR-CNM *BIN1* related may show normal to slightly elevated CK (54,55). Electromyography may be normal (55), although pseudomyotonic and myotonic discharges, myopathic muscle action potentials and fibrillations were seen (54). To note that electrical myotonia was not associated with clinical myotonia (54). A decremental response to repetitive nerve stimulation explained fatigability in Clayes et al (54) patient.

Biopsy leads to high suspicion of CNM *BIN1* related by the presence of one nucleus per fiber and mainly cluster of centrally placed nuclei in most muscle fibers and predominance of type 1 fibers (1,54). Type 1 fiber uniformity was observed (56). More rarely, disorganization of myofibrillar texture, dilated T-tubules and radial sarcoplasmic strands are encountered (54). In a severe case muscle adiposity and increase of connective tissue was seen (56).

Molecular genetics unrevealed homozygous BIN1 c.105G>T, c.451G>A

and c.1723A>T mutations, the latter introducing a premature stop codon. In one case Clayes (54), a homozygous missense mutation occurred in exon 6.

AD-CNM-*BIN1* related laboratory findings included normal or elevated (4 to 10 times the upper limit) CK; myopathic pattern in the EMG and muscle biopsy similar to AR-CNM--*BIN1* related, including clusters of central placed nuclei (56).

In brief, muscle biopsy with characteristic central nucleus and clustering of nuclei is the main features that raise the hypothesis of *BIN1*-CNM. After, a NGS of *BIN1* may confirm the inheritance, either AR or AD and the exact type of mutation.

Muscle RM in *BIN1* mutation was displayed by Clayes et al. (54) and CT scan in one of the family members in Kouwenberg et al (58) report. In the latter, the main feature was bilateral fatty replacement of the muscles of the lower back, and hamstring muscles, with sparing of the biceps femoris. Also, fatty replacement was present in the tensor fascia lata, adductor magnus, and in the muscles of the posterior compartment of the lower legs.

***BIN1* gene and its coding proteins**. *BIN1* gene (MIN*601248), also known as AMPH2 (amphiphysin 2) or AMPHL (amphiphysin 2-like), comprises 20 exons on chromosome 2q14.3 having a SH3 domain important for interaction with DNM2 protein and, a P domain. Due to alternative splicing of BIN1, at least 10 isoforms of the protein may be seen Nicot et al (13).

Autosomal recessive and autosomal dominant rianodine related centronuclear myopathy (*RYR1*-CNM-AR and *RYR1*-CNM-AD)

Mutations in the skeletal muscle ryanodine receptor 1 (*RYR1*-MIN*180901) gene are associated with a wide range of phenotypes, comprising the malignant hyperthermia susceptibility trait, and core myopathies, including central core disease and multiple minicores (59).

In 2007, Jungbluth et al (12) reported de novo dominant *RYR1* mutation in a sporadic case of myopathy with neonatal presentation of symptoms. They considered the RYR1 gene as a candidate for CNM because the observed clinical and histopathologic overlap between CNM and *RYR1*-related myopathies, along with an evocative and selective involvement on muscle MRI.

External ophthalmoplegia is a clinical feature present in a proportion of cases with AR mutations in the *RYR1* gene (2, 62). Wilmshurst et al (62) and

Bevilacqua et al (60) reported clinical, histological and molecular characterization of patients initially diagnosed with CNM due to the significantly high number of fibers with internalized nuclei. *RYR1* recessive mutations were found in every patient of the series.

Histopathological features range from normal muscle to type 1 fiber predominance with or without cores, an increased number of centrally located internal nuclei, and variable degrees of fibrous and adipose tissue (1, 60,62).

Biopsy in H&E and NADH-TR shows type 1fibers with hypotrophy fibers, the central nuclei are present in several of the hypotrophic fibers and the longitudinal sections have shows that they are spaced by the center of the fibers. Also, there is a central accumulation of oxidative enzyme stains, no radial distribution of the sarcoplasmic reticulum, and not excess of connective tissue or necrosis. Oxidative enzyme (COX) stains show central loss of stain, resembling central cores (12, 61).

MRI in the thighs show diffuse involvement of the quadriceps with relative sparing of the rectus femoris compared to the vastus intermedius, and of the gracilis compared to the sartorius. Within the lower legs, there was diffuse involvement with relative sparing of the gastrocnemii compared to the soleus, and of the tibialis posterior compared to anterior compartment muscles (12). This pattern of selective involvement was almost identical to that observed in patients with multiple cores and ophthalmoplegia due to recessive mutations in the RYR1 gene (63).

Autosomal recessive titin-related centronuclear myopathy (*TTN*-CNM-AR)

Ceyhan-Birsoy et al (24) discovered *TTN* gene mutations in patients that had clinicopathological CNM diagnosis. The authors used whole-exome (4 cases) or -genome sequencing analyses (one case). They identified all patients as compound heterozygous for mutations in *TTN* gene. Mutations resulted in truncated or disrupted skeletal muscle isoforms of titin protein.

All patients were male, and none had ocular involvement, three high arched palates and four facial weaknesses. One child had reduced fetal movements, two weakness and respiratory insufficiency since birth; one head lag and delayed motor milestones presented at five months. In two cases muscle deficit was detected at three years-old. All five patients had diffuse weakness associated with

areflexia. Four of then presented scoliosis, two associated with decreased vital capacity and two using respiratory support. None had overt cardiac involvement.

Fattori et al (64) identified one case with *TTN* mutation in a cohort of 54 Italian cases of CNM. Although *TTN*-CNM is very rare, precise genetic diagnosis is very important because regular cardiac monitoring of patients is obligatory to prevent morbidity and sudden death, considering the high frequency of cardiomyopathy and arrhythmias due to *TTN* mutations.

Titin is a giant sarcomeric protein placed from de Z disc to the M band and is codified by *TTN* gene, on chromosome 2, that has 363 exons. The protein interacts with nebulin and calpain 3 and has no direct function on excitation-contraction coupling apparatus (15), the contrary that occurs with proteins codified by gene (*RYR1*, *SPEG*, *MTM1, DNM2*) mutations associated with CNM.

Autosomal recessive centronuclear myopathy *SPEG* related (*SPEG*-CNM-AR).

In 2014, a new cause of CNM was discovered, homozygous or compound heterozygous mutations in the *striated muscle preferentially expressed protein kinase complex locus* (*SPEG* complex*)* (16). Three cases were published, one issued from a consanguineous parent and two from unrelated ones (16). The clinical main features were severe congenital myopathy associated with dilated cardiomyopathy in 2/3 patients. Interestingly, microstomia, retrognathia or retromicrognathia were observed. Recent publication (65) added two cases of congenital onset, one developing cardiomyopathy. In the total, dilated cardiomyopathy is the key to suspect of *SPEG* mutation among the clinical heterogeneous group of CNM.

Histopathology revealed unique centralized nuclei in most myofibers in all patient, few necklace fibers in one, hypotrophic fibers in two and fiber 1 predominance in one (16, 65). A reduction or absence of SPEG alpha and beta isoforms was detected in two cases using Western blotting (16).

SPEG complex locus and its coding proteins. In humans, *SPEG* complex locus is located on chromosome 2q35 and has 50 exons, coding the SPEG protein. The gene has several immunoglobulin domains (Ig domains), two fibronectin III (Fb) and and one protein kinase (Pk) domains. The Fb domain is important for interaction with myotubularin (16, 66). SPEG protein plays a role in

the excitation-contraction junctional activities.

In the total, to think a CNM diagnosis we must have a consistent argument based in the balance of clinical “phenotype up” and “phenotype down” (67) added by specific anatomopathological features, in conjunction with muscle MRI. When all evidences are congruous, molecular genetics tests is indicated to search a definitive diagnosis. Nevertheless, interpretation of molecular genetics exams with expertise is recommended to avoid equivoques.

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3.0 OBJETIVOS

3.1 Geral

Conhecer os aspectos funcionais e de imagem numa coorte de pacientes com MCN.

3.2 Específicos

Identificar possíveis padrões de alteração em grupos musculares através da Ressonância Magnética muscular.

Avaliar a função motora dos pacientes através da escala MFM-P.

Descrever casos incomuns da coorte de MCN, em relação a aspectos anatomopatológicos e/ou de genética molecular.

4.0 METODOLOGIA

Desenhou-se estudo observacional e descritivo incluindo pacientes com MCN matriculados no Ambulatório de Doenças Neuromusculares do HC-FCM UNICAMP, selecionados a partir do banco de biopsias musculares incluindo pacientes de qualquer faixa etária, além de pacientes cujo diagnóstico foi realizado durante o estudo, seja através da biopsia de músculo ou por genética molecular. O estudo recebeu a aprovação do Comitê de Ética e pesquisa da FCM UNICAMP sob o número 834.006, CAAE: 26988114.1.0000.5404

4.1 Critérios de Inclusão

Foram avaliados aqueles pacientes que convidados a participar do estudo, inteiraram-se do mesmo, tiveram suas dúvidas esclarecidas, conhecimento das etapas de avaliação e assinaram o termo de consentimento livre esclarecido. Os pacientes menores de idade foram representados pelo seu responsável legal.

4.2 Critérios de Exclusão

Pacientes que não concordaram em assinar o termo de consentimento livre e esclarecido ou que faltaram em avaliações de MFM-P e/ou RM de músculo.

4.3 Avaliação Funcional

4.3.1 Escala Medida da Função Motora (MFM-P)

A escala MFM, Validação para o português do Brasil (Escala Medida da Função Motora versão em português - MFM-P) analisa as funções da cabeça, tronco, segmentos proximais e distais de membros, em 32 itens, incluindo avaliações estáticas e dinâmicas, dividida em três dimensões: Dimensão 1 (D1): posição em pé e transferências, com 13 itens; Dimensão 2 (D2): função motora axial e proximal, com 12 itens; Dimensão 3 (D3): função motora distal, com 7 itens, dos quais 6 são referentes aos membros superiores. Para cada item é feita a graduação em uma escala de 4 pontos (escores de 0 a 3). Escore 0 - não pode iniciar a tarefa solicitada ou não pode manter a posição inicial. Escore 1 – esboça o item. Escore 2 – realiza parcialmente o movimento solicitado ou o realiza completamente, mas de modo imperfeito. Escore 3 - realiza completamente o

item, com movimento controlado. O escore total e de cada dimensão são expressos em porcentagens em relação ao escore máximo (96 pontos) [22-23].

4.4 Imagens de RM muscular (RMm)

Imagens obtidas em aparelho Philiphs 1.5 T, do Departamento de Radiologia da FCM-UNICAMP, com a colaboração de profissional técnica, em protocolo idêntico para todos os casos, segundo proposto por DeCAUWER *et al* [25].

As imagens foram ponderadas em T1w (TR = 500 ms e TE = 20 ms) e adquiridas usando uma seqüência SE (spin echo), sem a injeção de contraste paramagnético, com matriz de 256 x 256 e FOV (field of view) variável de 25 – 50 cm. Foram realizados 10 cortes de 5 mm de espessura, com espaçamentos variáveis de 10 a 50 mm, na dependência da extensão da região a ser examinada no plano axial, nas regiões: 1) Região cervical: imagens ponderadas em T1 obtidas desde o osso ióide (nível C5-C6) até o corpo vertebral da segunda vértebra torácica. Bobina de coluna cervical. 2) Ombros: imagens ponderadas em T1 obtidas desde o limite superior do corpo vertebral de C7 até o ápice da axila, correspondendo ao nível de bifurcação da traquéia. Bobina de corpo. 3) Braços: imagens ponderadas em T1 obtidas do ápice da axila até a junção entre o terço médio e distal do braço. Bobina de corpo. 4) Pélvis e coxas: imagens ponderadas em T1 obtidas do teto do acetábulo até a junção entre o terço médio e distal da coxa. Bobina de corpo. 5) Pernas: imagens ponderadas em T1 obtidas de ambas as pernas, de proximal (5 a 10 cm abaixo da articulação patelo-femoral) para distal (5 a 10 cm acima do nível dos maléolos. Bobina de crânio.

As imagens foram avaliadas por radiologista, sem conhecimento da clínica, e classificadas de acordo com o grau de degeneração muscular proposto por Mercuri *et al* [21]: grau 0 (músculo normal); grau 1, 2a, 2b, 3 e 4 (músculo com lipo-substituição completa). Aos graus acima foram atribuídos os valores zero; 1; 2; 2,5; 3 e 4 respectivamente.

4.5 Biopsia Muscular

As biópsias foram realizadas com técnica aberta e o músculo foi armazenado em nitrogênio líquido e fragmentos de 8 mm foram então obtidos para análise histológica. Amostras de músculo foram montadas em lâminas,

posteriormente coradas com hematoxilina e eosina, tricrômico de Gomori modificado, ácido periódico de Schiff e *oil red O*. Os especimens foram submetidos a reações com enzimas nicotinamida adenina dinucleotídeo tetrazólio redutase e succinato desidrogenase e imunohistoquímica para miosina lenta e rápida. As técnicas observaram as recomendações de Dubowitz *et al*[26].

4.6 Exame de DNA para Genética Molecular

Amostra de sangue periférico foram retiradas por punção venosa dos pacientes em laboratórios especializados, fora da UNICAMP. Captura de exons com *Nextera Exome Capture*, seguida por sequenciamento de nova geração (NGS) com Illumina HiSeq. Alinhamento e identificação de variantes utilizando protocolos de bioinformática, tendo como referência a versão GRCh37 do genoma humano. Análise médica orientada pelas informações que motivaram a realização do exame.

5.0 RESULTADOS

5.1 CAPÍTULO 2 – MIOPATIA MIOTUBULAR

Submetido Jornal de Pediatria em 14/12/2017

Manuscript Details

Manuscript number	JPED_2017_963
Title	Myotubular myopathy: case series with one patient with a novel mutation and other with contiguous genes syndrome
Short title	Myotubular myopathy
Article type	Original article

Abstract

Objective: To describe four cases of myotubular myopathy emphasizing uncommon clinical and genetic findings in two patients. Methods: Muscle biopsies were the golden standard in the diagnosis of two patients. One had biopsy, molecular genetics and muscle magnetic resonance. The other had DNA analysis by new generation sequencing. Results: Two male patients had classical phenotype including severe diffuse hypotonia and paralysis, feeding and ventilatory support since birth and a prolonged intensive care unit assistance, although one fatal case. In one patient the observation of hypospadia, thin and short penis and cryptorchidism besides the myopathy raised the hypothesis of a contiguous gene syndrome (MTM1/MAMLD1) confirmed by molecular genetics. One patient with severe myopathic phenotype had a novel pathogenic variant (c.482_485delTGGA).Conclusions: Myotubular myopathy is a severe congenital myopathy that affects male neonates, imposing pediatric intensive care assistance and further multidisciplinary coordinated approach to maximize survival and quality of life. Genital and hormones abnormalities may raise the possibility of contiguous gene syndrome, further defined by DNA analysis. In clinical highly suspect cases of myopathy, new generation sequencing of DNA favor definition of myotubular myopathy without the need of invasive procedures.

Keywords	centronuclear myopathy; contiguous gene syndrome; MAMLD1 gene; MTM1 gene; muscle magnetic resonance imaging; myotubular myopathy
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Myotubular myopathy: case series with one patient with a novel mutation and other with contiguous gene syndrome

Miopatia miotubular: serie de casos com um paciente apresentando nova mutação e outro a síndrome de genes contíguos.

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Keywords: centronuclear myopathy, myotubular myopathy, contiguous gene syndrome, *MAMLD1* gene, *MTM1* gene, muscle magnetic resonance imaging,
Running title: Myotubular myopathy
Acknowledgment: We wish to express our gratitude to the patients and their parents.TSR is supported by CAPES Foundation, Ministry of Education, Brazil.
Conflict of interest: the authors declare no conflict of interest.

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Abstract.

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Methods: Muscle biopsies were the golden standard in the diagnosis of two patients. One had biopsy, molecular genetics and muscle magnetic resonance. The other had DNA analysis by new generation sequencing.

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Conclusions: Myotubular myopathy is a severe congenital myopathy that affects male neonates, imposing pediatric intensive care assistance and further multidisciplinary coordinated approach to maximize survival and quality of life. Genital and hormones abnormalities may raise the possibility of contiguous gene syndrome, further defined by DNA analysis. In clinical highly suspect cases of myopathy, new generation sequencing of DNA favor definition of myotubular myopathy without the need of invasive procedures.

Centronuclear myopathies (CNM) are rare and heterogeneous group of inherited disorders with diversity in clinical presentation and genetic etiology (1). According to timely order of genetic discoveries in CNM, we may list: X-linked myotubular myopathy (XLMTM) (2); autosomal dominant (AD)-CNM-*DNM2* related (3); AD-CNM-*RYR1* related (4); autosomal recessive (AR)-CNM-*BIN1* related(5); AD-CNM-*BIN1* related (6), AR-CNM-*TTN* related (7) and CNM-*SPEG* related (8).

The most severe and fatal disorder of CNM's is XLMTM, for which no current cure exists. This disorder may affect males since gestation or birth and is associated with life-threatening events that often lead to prolonged pediatric intensive care admission. XLMTM was described in 1969 in a large Dutch family (9) and in 1990 the gene locus was defined at Xq28 (10,11). In 1996, Laporte et al (2) identified *MTM1* as the causative gene, which encodes the myotubularin protein.

We aim to report a case series of XLMTM patients from a tertiary university

hospital diagnosed during 1999-2016. We highlight one patient bearing the contiguous gene syndrome in whom muscle magnetic resonance image (MRI) was performed and other patient with a novel *MTM1* gene mutation.

Methods

This study was approved by the Ethics Committee of our institution and a written informed consent was obtained by the legal representatives of patients.

Selection of patients: from a biopsy bank we retrospectively reviewed 02 XLMTM-cases, along with the patients' medical charts. Two recent patients had genetic confirmation and are in clinical follow-up.

Biopsies were performed using an open technique and muscle was stored in liquid nitrogen and fragments of 8 mm were then obtained for histological analysis. Muscle samples were mounted on slides, which were subsequently stained with hematoxylin and eosin, modified Gomori trichrome, periodic acid-Schiff and oil red O. They were submitted to reactions with reduced nicotinamide adenine dinucleotide tetrazolium reductase and succinate dehydrogenase enzymes, and immunohistochemistry for fast and slow myosin ATPase (12)

Samples of peripheral blood were taken from patients for DNA analysis. Two patients underwent whole exome sequencing using Nextera® capture kits and sequencing was performed on aIlumina HiSeq 2500 platform®.

Muscle MRI was performed in a 1.5 Tesla scanner with acquisition parameters following those published by Mercury et al (13). T1-weighted axial images were obtained from pelvis to the ankles.

Results

Patient 1. A male patient was born from young and non-consanguineous parents after 38 weeks of gestation. He had decreased fetal movements, first minute Apgar equal to zero and 2, at 5 minutes of life; severe weakness and hypotonia; and immediate need of invasive ventilation. The patient needed continuous ventilatory support ever since. We have first seen the patient when he was 6 months old. There was intense hypotonia, decreased spontaneous movements, cephalic perimeter of 43.5 cm and feeding through gastrostomy. Cryptorchidism and high arched palate were also noticed. Maximal motor acquisition was seating without support that occurred when he was 19 months old. Muscle biopsy was

performed at 8 months old (year 1999) and is shown in Figure 1a. The patient received multiple professional cares but died at 2 years and 9 months due to septicemia from pulmonary infection. A genetic molecular diagnosis was not available at the time and the parents decided not to have other children.

Patient 2. The first male child of normal and unrelated parents was born with severe hypotonia, skeletal muscle weakness and poor sucking. He needed artificial ventilation soon after birth and remained in intensive care unit for long time with nutritional support through gastrostomy. The mother mentioned decreased fetal movements, but pregnancy was otherwise uneventful. He came to our institution for a muscle biopsy at 14 months of age (Figure 1b).The patient was lost from follow-up, but we were informed that he was ultimately discharged for home care after prolonged hospitalization. He survived until adolescence.

Patient 3. A male child born in 2013 from young and unrelated parents had a history of transient polihydramnious. He was delivered by cesarean section after 38 weeks of gestation; weighted 2.900 g, measured 51 cm and had immediate Apgar score of 2 and, 5 minutes later, score 3. He had cardiac arrest successfully reverted and 24 hours of ventilation support was seated. Intense hypotonia, severe generalized weakness and feeding difficulties were present for months. An extensive laboratory investigation to elucidate the etiology of his condition included routine search for congenital infections and inborn errors of metabolism, cariotype, cerebral computed tomography and creatinekinase (CK), but was unremarkable. When he underwent surgical gastrostomy, muscle samples of the rectus abdominis were also obtained for histological analysis (Figure 1c and d). He was first seen in our institution at 10 months and presented severe hypotonia, a high arched palate, dolichocephaly, reduced external ocular movements, mild bilateral ptosis and bilateral cryptorchidism. The child slowly evolved with motor achievements and ultimately needed only nocturnal BIPAP®. At age 3-year-old, he gained cervical control and was able to speak using a speak valve. He can seat with mild support and is under intensive physical rehabilitation. Because hypospadia [type non-severe, subcoronal (14)], thin and short length penis (<3 cm) and cryptorchidism (Figure 2) hormonal screening was indicated. It showed decreased dehydroepiandrosterone, free testosterone and total testosterone levels. Abdominal ultrasonography confirmed bilateral undescended testicles with normal glands appearance. Molecular genetic analysis revealed a large deletion

in Xp28 [ChrX:149.613.732-150.156.389], including the *MTM1* gene and partially the gene *MAMLD1* (*Mastermind-Like Domain Containing 1*). MRI of the patient is displayed in Figure 3. His mother defines herself as a healthy woman and despite this, we recommended her to undergo either a CGH or SNP-array to guide proper genetic counseling. She has not yet decided about pursuing genetic testing.

Patient 4. A 6 months old boy was the second child of young, non-related and healthy parents and had a normal six-years-old sister. In the 7th month of pregnancy, polihydramnious was detected, but the mother denied decreased fetal movements. After a term gestation, he was delivered by cesarean section in 2016, weighing 2.615 gand measuring 51 cm. His Apgar scores were 1; 5 and 7, respectively at one, five and ten minutes, resulting in intensive care hospitalization for 03 months after which he was discharged for home care, after traqueostomy and gastrostomy. An extensive investigation (analysis for congenital infections, inborn errors of metabolism, CK, cariotype, echocardiography and abdominal ultrasonography) was unremarkable. A cerebral MRI revealed abnormal bilateral ventrolateral thalamic signals, compatible with anoxic-ischemic injury. Neuromuscular exam at 6 months revealed cephalic perimeter=43.5cm, biauricular=25cm, anteroposterior=27 cm; mild *pectus carinatum*, undescended testicles, severe axial-proximal hypotonia and paralysis, mild facial weakness and external ophthalmoparesis, abolished muscle stretch reflexes. Whole exome sequencing revealed the c.482_485delTGGA variant in hemizygosis at *MTM1*. At age 18 months the child uses nocturnal VPAP®, is feeding by gastrostomy and has intense physiotherapeutic care. He can stay in sitting position without support and straightened head.

Discussion

We present four XLMTM-patients, two diagnosed by clinic and pathological features. One had fatal outcome, other survived after intensive multidisciplinary healthy approach. We currently see two of the patients. These had peculiar genetic findings: one had contiguous genes syndrome (*MTM1* and *MAMLD1)* and the other had a novel mutation in *MTM1* gene. Although our case series of XLMTM is small, it is representative of a tertiary university hospital, especially because the myopathy seems to be rarer in Brazil (15).

The neonatal period of life is the most critical for XLMTM-patients (16) due to their inability to maintain unassisted respiration and feeding as occurred with all our patients. Generally, the disease is fatal in early infancy (16), like in case 1. Survival rates ameliorate with increasing age (16), but this is partially consequence with maximal effort to provide excellent care.

Clinical phenotype of our patients was classical for XLMTM (1,15,16) and in combination with histopathology (cases 1-3) raised the diagnosis. Myopathology is characterized by central positioning of a large nucleus in variable number of rounded muscle fibers, either type 1 or type 2 (17) as we showed in our patients. In some myofibers, it may be noticed a central hole devoid of myofibrils. Additional features include accumulation of oxidative enzymes in the center of the fiber and pale peripheral halos (17), predominance of type 1 fibers and necklace fibers (18). The later were seen in neonatal severe cases and also in mild and late-onset XLMTM, in a proportion of 3 to 100% of myofibers (18,19) and may also be seen in CNM with *DNM2* mutation (20)

Cryptorchidism was observed in cases 1, 3 and 4 and is a frequent finding in XLMTM (1,13). However, patient 3 had also mild hypospadia, micropenis and chryptorchidism, altogether suggestive of abnormal sexual development and a contiguous gene syndrome (21). Indeed, molecular genetic analysis in this patient confirmed a large deletion in *MTM1* and partial deletion in *MAMLD1* gene. The discovery of *MALMD1* related to sexual abnormalities was possible in the context of case studies of XLMTM (14,22). *MAMLD1* mutations cause hypospadias primarily because of compromised testosterone production around the critical period of sex development (22) and in our case 3 the hormonal level was low.

Muscle CT or MRI was done in a restricted number of subjects with adult-onset *MTM1*-related myopathy (19,23). The main features observed by the authors (19) were volumetric asymmetry of lower limbs with atrophy and fat infiltration of pelvic and thigh muscles. Drouet's (23) adult female patient had left-side predominant symptomatic myopathy, corresponding to MRI abnormal images.

To the best of our knowledge, the assessment of muscle-MRI in male congenital-onset XLMTM as in our patient 3 was never published. MRI was performed early in life and proved helpful in the rehabilitation planning. Pelvis, thighs and legs muscles showed diffuse volumetric reduction and fat infiltration,

with predominance in legs and gluteal region, especially gluteus maximus. Muscle volume of posterior thigh was more affected compared to quadriceps, as occurred with fat infiltration. In the legs, gastrocnemius medialis was relatively spared.

A novel mutation in *MTM1* gene was detected in patient 4. Since 2012, over 400 mutations were described (24,25) more frequently in exons 4, 8, 9, 11 and 12. In about 7% of XLMTM patients large deletions occurred (24). The novel pathogenic variant c.482_485delTGGA, in ChrX: 149.807.452-149.807.456, *MTM1* gene was not detected in about 61.000 Brazilians and promotes substitution of tryptophan at position 164 for glutamine, creating a premature stop codon in protein translation 21 residues after (p.Trp164Glufs*21). This particular patient highlights the importance of molecular genetic testing since it may establish diagnosis with no need of invasive and risky procedures such as muscle biopsy.

In conclusion, the case series is representative of the rarity and severity of XLMTM in our country. Pediatricians and neonatologists should be aware of the disease to guide proper management early in life. Muscle biopsy still has a major role in the screening for congenital myopathies in our country. Nevertheless, advanced genetic testing will certainly help in the diagnostic assessment of such patients, as seen in our case with a novel *MTM1* mutation and in the case of contiguous syndrome *MTM1*/*MALMD1*. The later genotype may be clinically suspected by the association of cryptorchidism, hypospadia and low hormonal levels.

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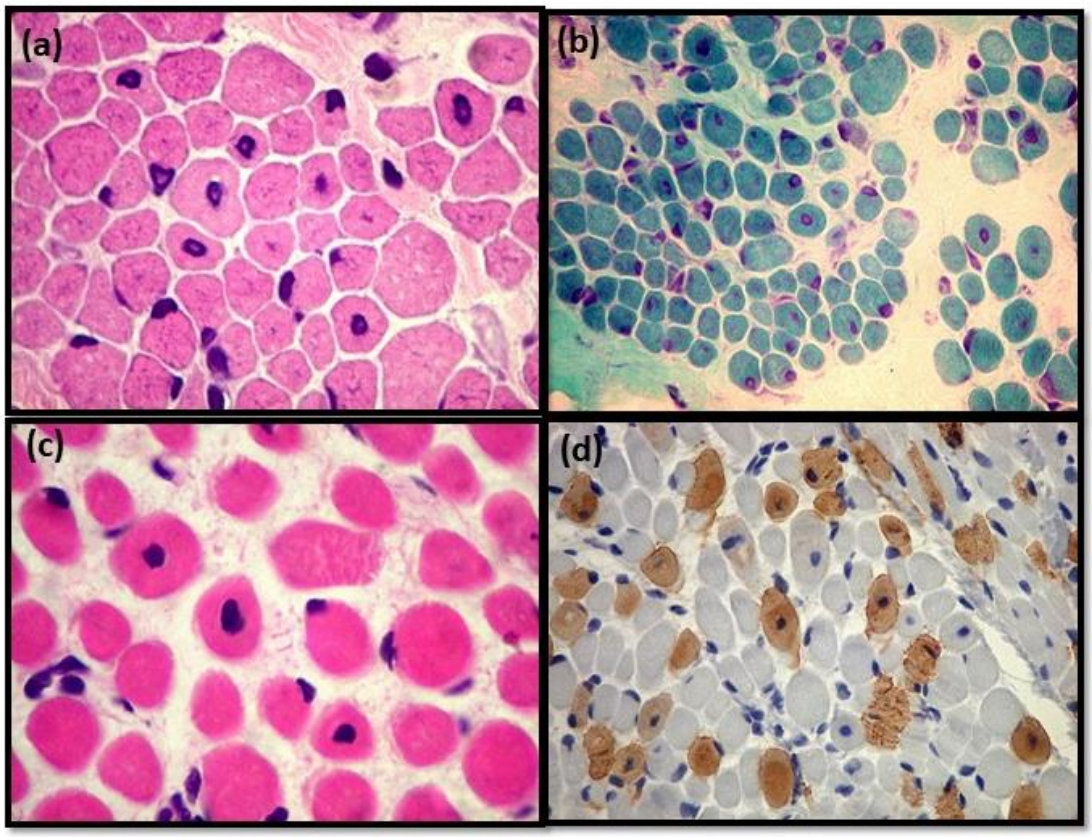

Fig 1. Histopathology: patient 1(a), patient 2(b), patient 3 (c and d). Transverse sections (a and c) hematoxylin and eosin staining, (b) Gomori trichrome, (d) fast myosin. Note several small fibers with large central nucleus. Type 1 fiber predominance is observerd in (d).

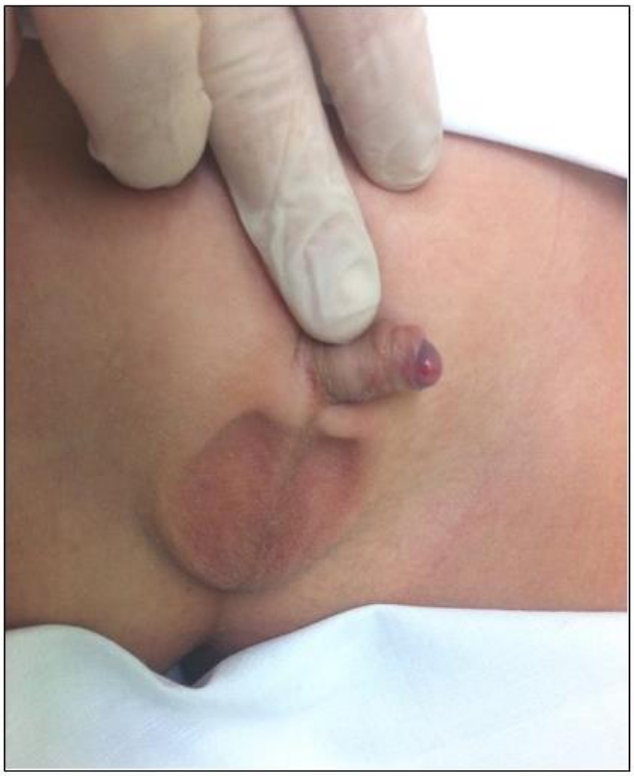

Fig. 2. Observe non-severe subcoronal hypospadias; short length penis (< 3 cm) and cryptorchidism in patient 3.

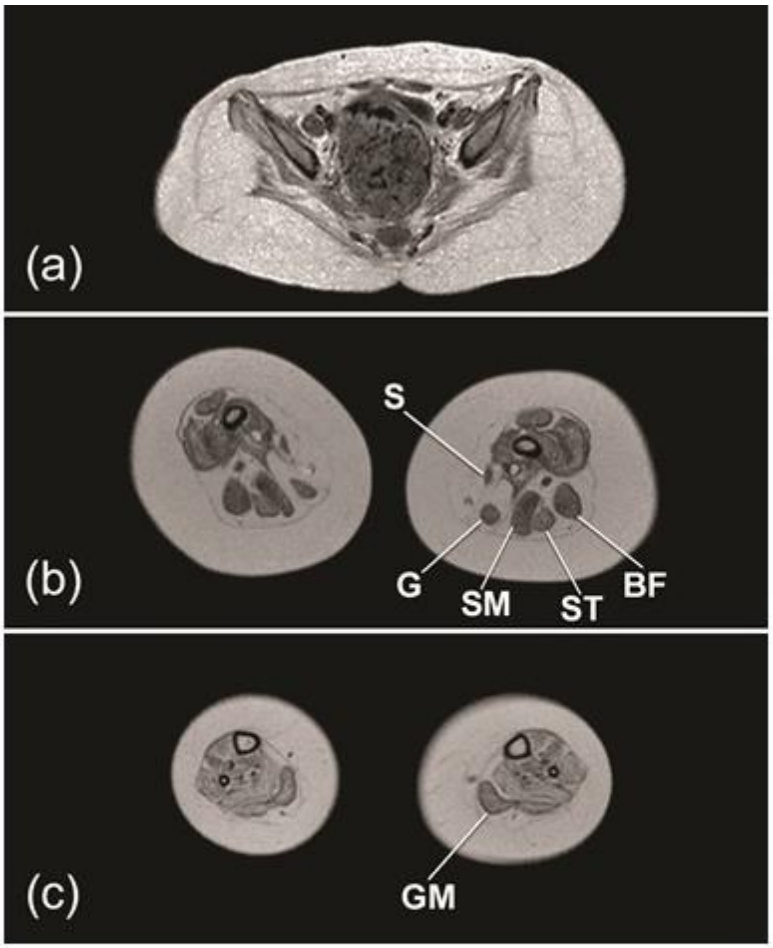

Fig 3. Axial T1-weighted MRI images of the pelvis (a), thighs (b) and legs (c) muscles are shown. There is a prominent fatty infiltration and atrophy of the gluteal muscles, particularly the gluteus maximum and a more homogenous involvement of the sartorius (S), gracilis (G), semimembranosus (SM), semitendinosus (ST) and biceps femoris (BF) muscles. In the legs, the medial head of the gastrocnemius (GM) is less affected than the others.

5.2 CAPÍTULO 3 - Miopatia congênita centronuclear autossômica dominante por mutação *DNM2*

Centronuclear myopathy with BIN1-like myopathology and DNM2 mutation. Expanding morphological phenotype of DNM2-CNM.

Miopatia centronuclear com aspecto miopatológico de mutação *BIN1* e mutação no gene da *DNM2.* Expandindo o fenótipo morfológico da MCN por mutação na DNM2.

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Abstract. *DNM2* gene mutations are frequent in centronuclear myopathies (*DNM2*-CNM) and the main biopsy characteristics is fibers with radiating sarcomeric strands along with nuclear centralization. **Objective**. To present a Brazilian case of *DNM2* mutation; muscle biopsy with clustering of muscle in several fibers, main features of *BIN1*-CNM histopathology. **Case report**. A 58-year-old woman was born as a “weak child” with “eyes closed”. She had reduced spontaneous movements, delayed motor milestones and a motor limitation life long. Recent complains were hypersomnia and cognitive impairment. Consultation revealed: bilateral symmetrical ptosis and external ophthalmoplegia; atrophy and motor deficit predominant at distal lower limb; pelvic more weak than scapular gilder. Motor functional measure scale had total score=65.62% and FVC=32%. Cognitive assessment (MoCA) was 22/30. Cerebrospinal fluid showed protein=82 mg/dL. Biceps brachii biopsy revealed several fibers with nuclear cluster centrally located and others with a central nucleus; type 1 fiber atrophy, type 2 hypertrophy and few fibers with radiating sarcomeric strands, thus predicting a CNM with *BIN1* mutation. Surprisingly muscle MRI was compatible with *DNM2*-CNM and a cerebral MRI disclosed meningioma. A pathogenic variant

(c.1105C>T) diagnosed *DNM2*-CNM. After a successful neurosurgery, the patient was unable of spontaneous ventilation, had septicemia and died. **Conclusion**. Our case expands histopathological phenotypes of *DNM2*-CNM and reinforce the features of pelvis and legs MRI in *DNM2*-CNM described in the literature.

Key words: Congenital myopathies, centronuclear myopathies, *BIN1*, *DNM2,* meningioma, histopathology.

Introduction

The *BIN1* gene may cause autosomal recessive (AR)[1] and autosomal dominant (AD) centronuclear myopathy (CNM) [2]. Until now a limited number of genetically proven cases of CNM-*BIN1* related have been described, in part because it accounts for about 5% of AR-CNM patients[3]. On the other hand, *DNM2* mutations are responsible for 50% of AD-CNM[4].

BIN1 codifies amphiphysin2 protein that have several important function in normal cell life and in diseases like CNM, myotonic dystrophy, Alzheimer disease and cancer[5] Interaction between amphiphysin2 and dynamin2 is necessary for normal muscle function and positioning of nuclei[1]. Interaction between amphiphysin2 and dynamin2 has been studied and both proteins are involved in plasma membrane tubulation required for T-tubule biogenesis [6].

We present clinical data, functional motor scale, muscle biopsy, muscle and cerebral MRI and molecular genetics of an interesting case with CNM clinical phenotype, benign tumors, including a meningioma and *BIN1*-CNM like histopathology. Surprisingly molecular genetics revealed a pathogenic variant in the middle domain of *DNM2*.

Case report.

A 58-year-old woman was the sixth child of non-consanguineous parents. Gestation and delivery was unremarkable and occurred in an ambience of a rural area. She was born as a “weak child” with the “eyes closed” and reduced spontaneous movements. Nevertheless, she walked at about two years-old, but had frequent falls. She never climbed trees, swimmer or runner as other children of her farmer’s family. At twelve years-old myopia and astigmatism was diagnosed and corrected by glasses that she ever uses. She chose a sedentary and intellectual lifestyle, attended at university and reached license to teach History at high school. However, she was unable to assume as docent because limbs weakness and low tone voice. At age 35, a thyroid nodule was removed by partial

thyroidectomy and ten years later she was submitted to Wertheim's hysterectomy, however without hormonal replacement. At age 41, neurological and neurophysiologic examination diagnosed Steinert disease, elsewhere. Electroneuromyography revealed normal conduction velocities; complex repetitive discharges (CRD) and myotonic discharges (MD) in muscles quadriceps, tibialis anterior, extensor digitorum and gastrocnemius. Polyphasic action potentials were registered in the mentioned muscles and also in biceps and triceps brachialis and deltoid. Repetitive stimulation was normal. Five years later, episodes of auditory hallucinations were frequent and a psychiatric prescription of haloperidol (2.5 mg/day) resolved the symptoms. The patient denied fatigability; swallow difficulty, sensory or sensitive symptoms and signaled sporadic episodes of cephalalgia. Her sister complained that more recently the patient spends longtime to performer routine tasks, has post-meals hypersomnia, but answered negative for memory or other cognitive abnormalities. Similar disease in the family was denied.

Our consultation revealed a patient using wheel chair for long distances and walking cautiously with short steps. She had a body mass index of 16.5, bilateral symmetrical ptosis and external ophthalmoplegia. Motor deficit was predominant at pelvic than scapular gilder and movements at ankle were graded 3/5. Stretch muscle reflex were diffusely hypoactive and no pathologic reflex was obtained. Motor Function Measure scale [MFM-P$^{(7)}$] had total score = 65.62% (D1=35.89%; D2=86.11%; D3=85.71%) and FVC = 32%. Montreal Cognitive Assessment (MoCA) was 22/30 and Epworth sleepiness scale 7. Laboratory investigation for hepatic, renal and thyroid functions, dyslipidemia, diabetes mellitus; serum lactic acid, creatine kinase, aldolase; electrocardiogram and echocardiogram were unremarkable. Holter revealed rarer atrial extrasystoles. Single fiber electromyography was normal. Cerebrospinal fluid showed protein of 82 mg/dL and Pandy positive. Biopsy of biceps brachii is displayed in Figure 1, muscle MRI in Figure 2 (b-h) and cerebral MRI in Figure 2a. NGS showed a pathogenic variant in Ch19:10.904.508C>T (c.1105C>T) diagnosing a CNM-*DNM2* related. After a successful neurosurgery, the patient never acquired spontaneous ventilation, as was suggestive by MFM and FVC, remained in intensive care unit for four months, had multiples episodes of septicemia and died.

Discussion.

We describe a patient with sporadic congenital-onset of CNM, slowly progressive, diagnosed initially by characteristic muscle histopathology. The patient had history of benign tumors in different tissues and a cerebral meningioma was documented by MRI, at the moment of investigation in our institution.

Important myopathic clinical features were ptosis and ophthalmoplegia, more frequently seen in CNM [8] AD-CNM-*DNM2*[9] and AR-CNM-*BIN1*[1]. Weakness was predominant in distal lower limbs in comparison to proximal and to proximal-distal upper limbs muscles, features described in AD-CNM-*DNM2* [4,9] and AD-CNM-*BIN1*[2].

Patient's examination by MFM scale revealed significant functional compromise, that is, fewer than 70% as expressed by the total score and a D1 dimension (standing and transfer tests) score of ≤40%. These numbers predicted loss of ambulation within one year in Duchenne dystrophy [10], but the literature is lacking in relation to similar correlation in CNM cases.

Previous EMG of the patient showed myopathic potentials, myotonic discharges (MD) and complex repetitive discharges (CRD). Neurophysiology and her phenotype resembling Steinert disease resulted in this misdiagnosis for 17 years, done elsewhere. In fact, myotonic dystrophy is a frequent neuromuscular disease and express MD and CRD, although differential diagnosis must be included. In CNM-*DNM2* genetic confirmed cases, MD was also registered (11) and CRD was more frequent compared to MD [12]. A patient with AR-CNM *BIN1*-related also presented CRD and MD [13]. Claeys et al [13] patient complained of weakness fluctuation that was correlated with fatigability by repetitive nerve stimulation. Our patient on the contrary had normal single fiber EMG.

Biceps brachii biopsy of our patient predicted a CNM with *BIN1* mutation because it showed several fibers with nuclear clustering centrally located and a large number of myofibers with one central nucleus. Type 1 fiber atrophy and type 2 hypertrophy and few fibers with radiating sarcomeric strands were also seen. In the total, they were histopathologycal peculiarities in accordance with genetic confirmed CNM-*BIN1* related cases [2, 13, 14, 15]. Morphological diagnosis in CNM-*DNM2* are done based in nuclear centralization more than nuclear internalization, vacuolization around central nuclei and fibers with radiating sarcoplasmic strand in NADH-TR, resembling "spoke of wheels", a hallmark of this subgroup of CNM

[14,16]. Necklace fibers were described in late-onset CNM-*DNM2*[17].

The pattern of myopathic distribution in the present case became evident in MRI: upper limbs less affected than lower limbs and thighs showing major muscle lipomatosis in posterior compartment and minor in the anterior. In this region, vastus intermedius was more affected of quadriceps. Legs had severe fat infiltration in all muscles, minor in tibialis posterior and extensor halux longus. Cervical and lumbar paravertebral muscles were also affected, in concordance with clinical observation of vertebral column rectification. MRI in scapular girdle and arms shows predominant fat infiltration in deltoid (anterior and medial) and short head of biceps braquial.

Muscle MRI in genetic confirmed cases of CNM-*BIN1* documented a severe involvement of gluteus minimus; posterior compartment of thighs, with some asymmetry and relative sparing of semitendinosus, whereas gracilis muscle was spared even in advanced stage of evolution. Medial head of gastrocnemius was initially and more severely affected, but with disease evolution, lateral head, soleus and peroneal were also involved [15]. The patient described by Clayes et al[13] had MRI showing very severe involvement of thighs and legs with gracilis and tibialis posterior relatively preserved.

MRI in CNM-*DNM2* genetically proved cases documented initial and most prominent involvement of tibialis anterior and soleus and gluteus maximus, whereas gracilis, sartorius and retus femoris were relatively spared even in severe cases [4,18, 19].

DNA analysis revealed a dominant mutation in exon 8, stalk (middle) domain of *DNM2,* resulting in a substitution of arginin to tryptophan (p.Arg369Trp) described before by Bitoun et al, 2005[9]. A total of 23 *DNM2* different mutations were listed by Hohendahl et al [6], since 2016. Exon 17 has more frequency of mutations followed by exon 8. These authors explored the consequences of mutations in the dynamin2 tetramer structure and interaction amphyphisin2-dynamin2.

Interestingly, the healthy history of our patient indicated different tissues exhibiting tumors, possibly benign, one in relation to thyroid gland, and other treated by hysterectomy and oophorectomy, according to patient's information. Complains of cephalalgia, hyperproteinorraquia and MoCa test were strong evidences for cerebral MRI exam indication. A brain tumor was uncovered by

cerebral MRI, showing images compatible with a right frontal meningioma that lead to a prosody and dysexecutive syndrome. Central nervous system involvement in CNM-*DNM2* mutation was described by Cateruccia et al[4] and expressed by epilepsy in two cases.

We may think the occurrence of myopathy and tumors in the patient were fortuitous. On the other hand, a very tempting option was the hypothesis that tumor growth was influenced by the same mutation that caused myopathy, if a *BIN1* mutation would be discovered. *BIN1* is important regulator in endocytosis and membrane recycling, DNA repair, cell cycle progression and apoptosis, thus linked to cancer progression[5]. Interaction dynamin2 and amphiphysin2 have been explored especially in view of tissue specificity, like muscle[6], and only few insight has been explored in relation to neural tissue[20].

In conclusion, our data expands morphological phenotypes of *DNM2*-CNM, reinforce the features of pelvis to legs MRI in *DNM2* mutation and adds novel knowledge about scapular and arms MRI. Anterior and medial deltoid and short head of biceps brachial exhibit more fat infiltration regarding other muscles.

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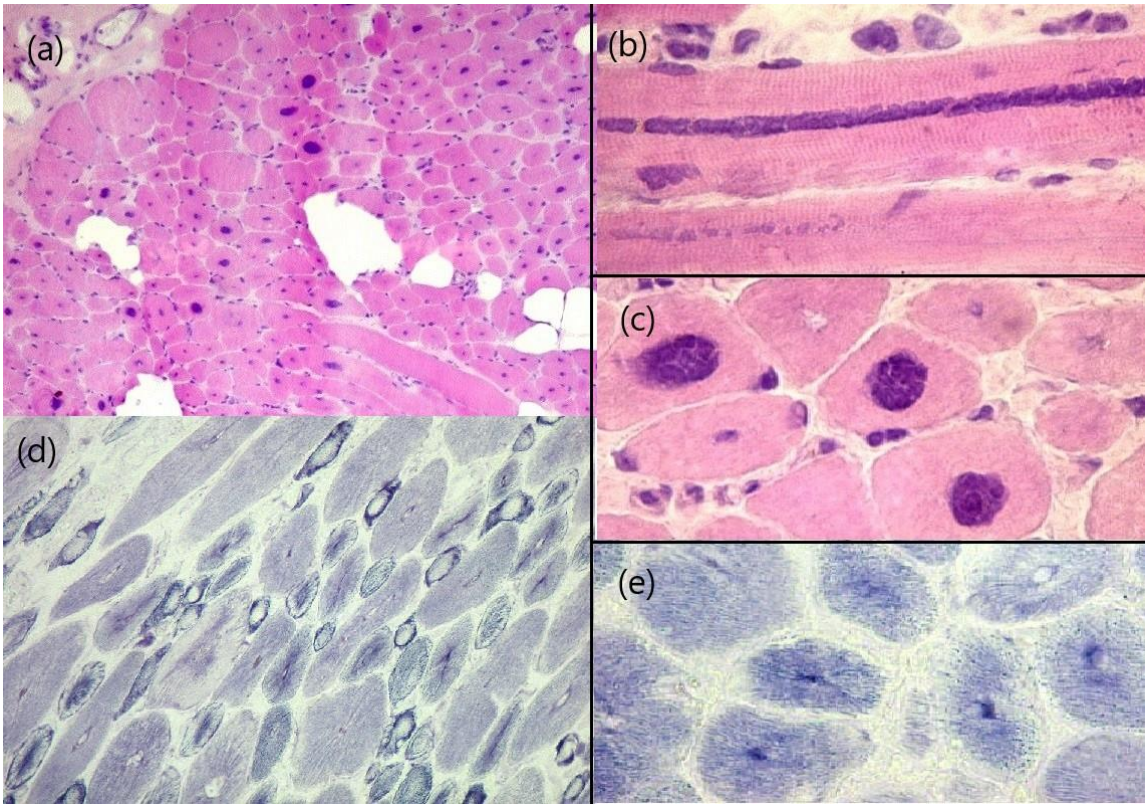
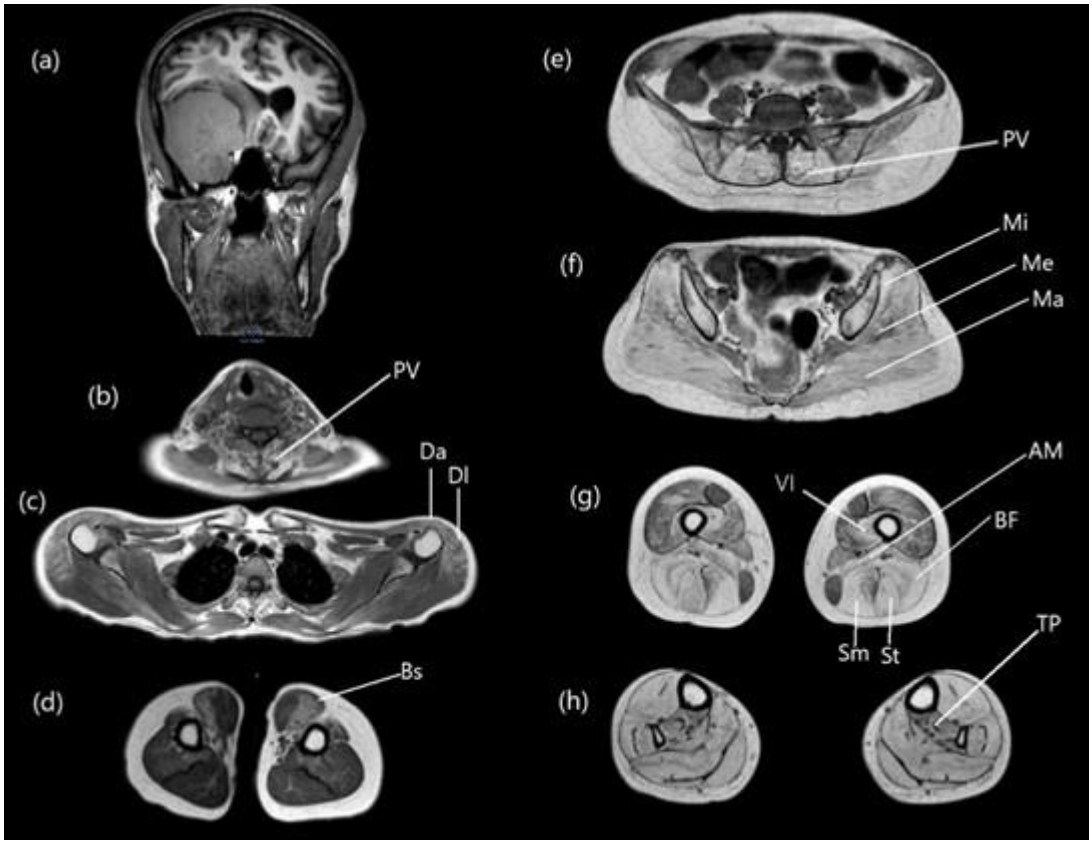
Figure 1: Morphologic features in biceps brachii biopsy



Legends: Biceps brachii biopsy. Haematoxilyn and eosin staining, a,b,c; transverse section (a and c) and longitudinal section (b). Note adipose cells in the sample and variation in fibers size; almost all muscle fibers have centralized nucleus and several fibers show clustering of nucleus. NADH-TR showing atrophy of type 1 fibers (d) and SDH (e) with few fibers with radiating sarcomeric strands.

Figure 2. Cerebral and Muscles MRI imaging



Legends: (a) cerebral MRI: T1-w image documents a large frontal tumor. (b-h) muscle MRI: T1-w images in transversal sections of cervical, scapular, arm, pelvis, thighs and legs. Note fat infiltration in cervical paravertebral muscles (PV, in b), deltoid (Da, Dl in c); bíceps brachii, short head (Bs in d), sacral paravertebral (PV in e), gluteus minimus (Mi) and maximus (Ma) more affected regarding gluteus medius (Me) in f. Vastus intermedius (Vl) and posterior compartment of thighs with major fat infiltration and relative sparing of retus femoris (F) and sartorius (S) in g. Tibialis posterior (TP) and extensor halux longus are less affected in the legs (h).

5.3 Capítulo 4. Resultados adicionais:

Imagem de músculo e miopatia centronuclear

INTRODUÇÃO

Imagens de músculos, mais especificamente de RMm, são obtidas através de exame não invasivo e tem sido auxiliar no diagnóstico de várias miopatias [1].

Em paciente com MCN-*DNM2*, Jeub et al.[2], encontraram infiltração gordurosa nos músculos glúteo máximo, bíceps femoral, adutor longo e em maior gravidade, no sóleo, gastrocnêmio medial e músculos do compartimento anterior das pernas, em particular no tibial anterior. Similar padrão foi observado por Susman e et al. [3] e Catteruccia et al.[4]. Não está bem definido padrões de comprometimento muscular em outros subtipos de MCN, entretanto a RMm poderá ser interessante ferramenta diagnóstica, por método não invasivo.

No capítulo apresentamos imagens de RMm e avaliação funcional, pela escala MFM-P, de 10 casos de MCN, sendo um paciente com confirmação genética para mutação no gene *DNM2.*

RESULTADOS

Na tabela 1 são apresentados dados sobre o perfil dos pacientes avaliados neste capítulo, informações como sexo, início dos sintomas e herança.

Tabela 1: Características demográficas da coorte com MCN.

Caso	***Sexo***	***Início dos sintomas***	***Herança***
F1-1	F	Adulto	AD
F1-2	F	Infância	AD
F1-3	F	Infância	AD
F1-4	F	Infância	AD
5	M	Congênito	Genética: mutação *DNM2*-AD
6	F	Congênito	Caso esporádico
7	F	Congênito	Caso esporádico
8	F	Congênito	Caso esporádico
9	M	Adolescência	AD *
10	F	Adulto	Caso esporádico

Legenda: F1 = família; * Pai falecido, com diagnóstico de MCN em outro serviço.

Na tabela 2, são apresentados os resultados da avaliação funcional de cada paciente, sendo que para 8 deles foi aplicada em 2 momentos, para 4 casos em 3 momentos e para um dos casos (F1-2) foi realizada em 4 momentos diferentes, no período de 4 anos. Em todos os casos a avaliação foi rezalida ao menos uma vez com score total médio de 64,26% na primeira avaliação. Observa-se que a dimensão de maior comprometimento funcional foi D1, com media de 33,32% nesta dimensão.

Vale resaltar que no caso F1-2 houve melhora do escore total da MFM-P, ao longo do período, em associação com a redução de peso corpóreo, a partir de correção nutricional, embora tenha havido piora na D1 (em pé e transferência) e melhora na D2 (função proximal axial). No caso F1-4 houve estabilidade funcional durante 25 meses.

A primeira avaliação da MFM-P, mais próxima da data do exame de RMm, foi de um mês e a mais distante ocorreu em períodos variáveis, com média de um ano.

Tabela 2. Datas e escores da MFM-P total e por dimensões em relação à data da RMm.

Caso	Data MFM	D1%	D2%	D3%	Total%	Data RMm (idade)	Data MFM	D1%	D2%	D3%	Total %	Data MFM	D1%	D2%	D3%	Total%	Data MFM	D1%	D2%	D3%	Total%
F1-1	06/14	38,46	94,44	95,23	71,87	08/15 (61)	07/15	28,2	88,88	80,95	62,5	07/16	28,2	88,88	80,95	62,5	-	-	-	-	-
F1-2	06/14	56,41	94,44	95,23	70,16	09/15 (35)	07/15	41,02	91,66	90,47	70,33	07/16	41,02	100	95,23	75	12/17	56,41	91,66	95,23	78,12
F1-3	06/14	41,02	100	95,23	75	08/15 (37)	07/15	41,02	91,66	90,47	70,83	07/16	38,46	91,66	85,71	68,75	-	-	-	-	-
F1-4	06/14	61,53	94,44	95,23	81,25	08/15 (33)	07/15	61,53	94,44	95,23	81,25	07/16	61,53	94,44	95	81	-	-	-	-	-
5	10/14	46,15	83,33	85,71	68,75	09/15 (16)	06/15	46,15	77,77	66,66	62,5	-	-	-	-	-	-	-	-	-	-
6	04/16	35,89	80,55	90,47	64,58	10/15 (9)	07/16	45,58	83,33	100	70,83	-	-	-	-	-	-	-	-	-	-
7	08/15	5,12	65,44	77,44	35	03/15 (34)	08/17	2,56	36,11	80,95	32,29	-	-	-	-	-	-	-	-	-	-
8	05/16	5,12	80,55	85,71	51,04	01/16 (43)	09/17	5,12	77,77	85,71	50	-	-	-	-	-	-	-	-	-	-
9	05/14	20,51	91,66	100	64,58	03/15 (42)	04/15	20	88,88	100	63,54	-	-	-	-	-	-	-	-	-	-
10	10/14	23,07	80,55	95,23	60,41	11/14 (61)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Média MFM		33,32	86,54	91,54	64,26			32,35	81,16	87,82	62,67		42,30	93,74	89,22	71,81					

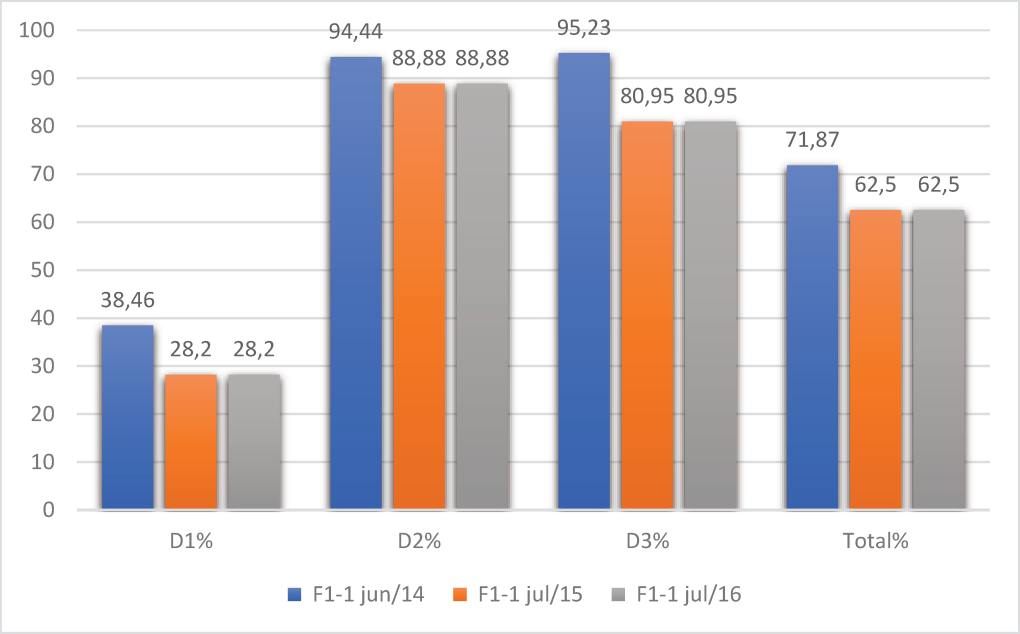
Legenda:F1=familia 1; MFM= Medida da Função Motora; D1=dimensão 1; D2 dimensão; D3=dimensão 3.
(idade)=(em anos);data MFM= mês/ano; data Rmn= mês/ano

OS Gráficos de 1 a 10 mostram a variação ou estabilidade funcional pela MFM-P.

A paciente refente ao caso 7 foi a mais grave da coorte, apresentando-se cadeirante e com grande comprometimento da musculatura, pondendo ser visto através das imagens de músculos (Figura 5), acompanhado de um importante comprometimento da MFM-P (gráfico 7).

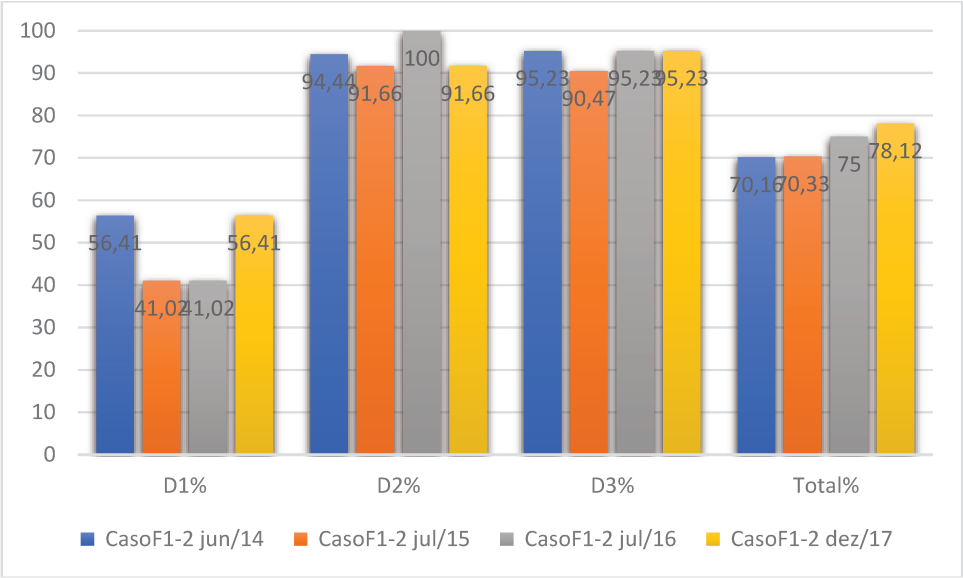
Para o caso 10, foi aplicada a escala MFM-P apenas uma única vez (gráfico 10).

Gráfico 1. Evolução MFM-P Caso F1-1



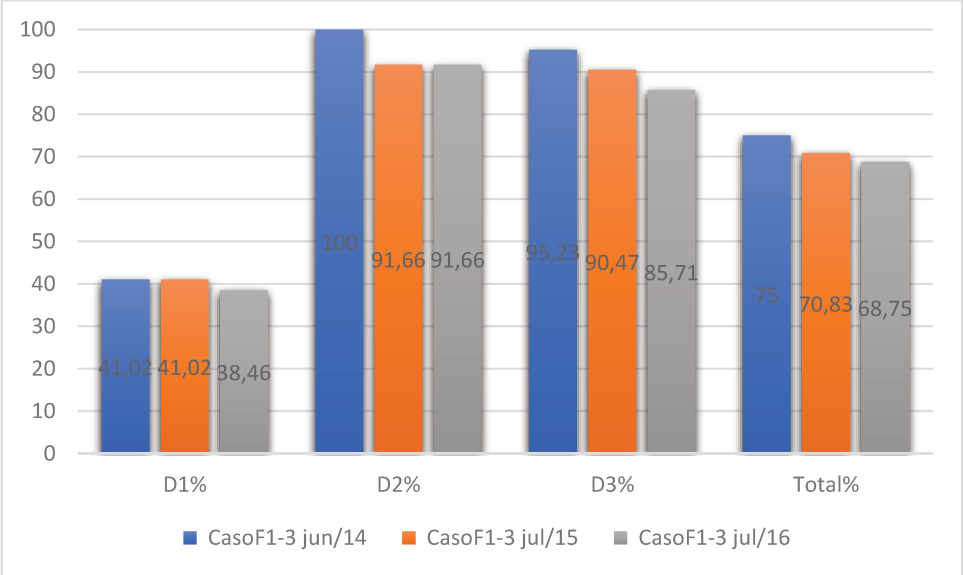
Legenda: F1=familia 1; as cores são referents ao ano de avaliação
Eixo y: números em %

Gráfico 2. Evolução MFM-P Caso F1-2



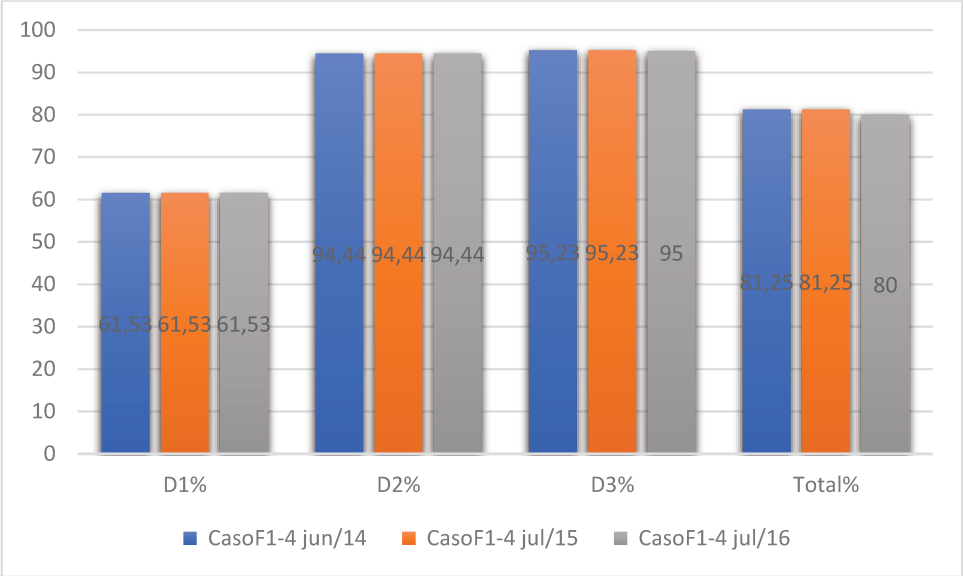
Legenda: F1=familia 1; as cores são referents ao ano de avaliação
Eixo y: números em %

Gráfico 3. Evolução MFM-P Caso F1-3



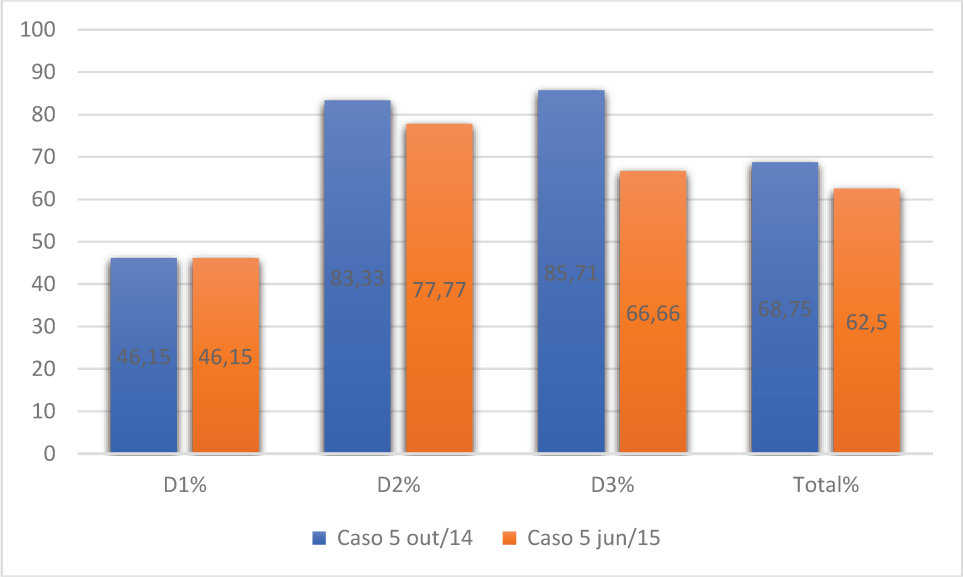
Legenda: D1=dimensão 1; D2 dimensão; D3=dimensão 3; as cores são referents ao ano de avaliação
Eixo y: números em %

Gráfico 4. Evolução MFM-P Caso F1-4



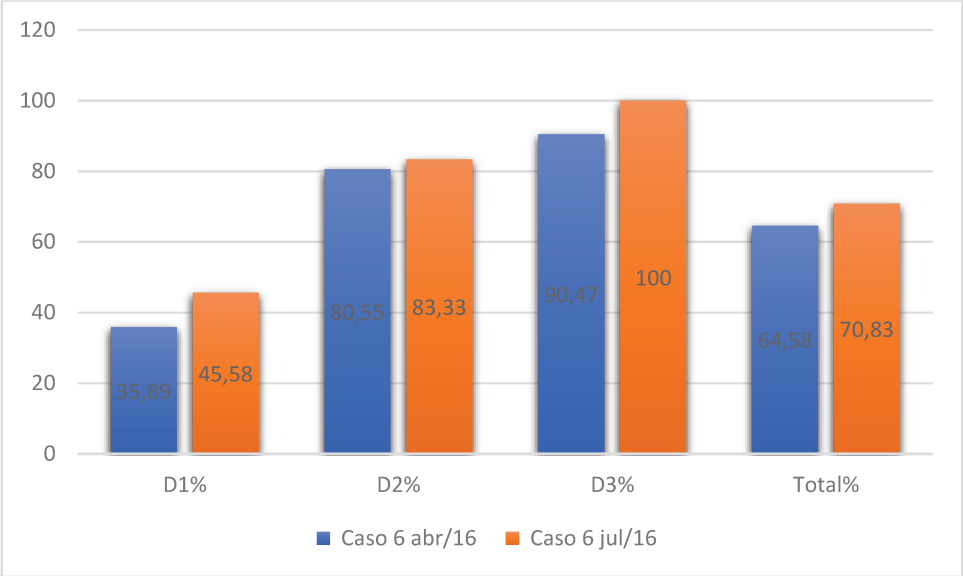
Legenda: D1=dimensão 1; D2 dimensão; D3=dimensão 3; as cores são referents ao ano de avaliação
Eixo y: números em %

Gráfico 5. Evolução MFM-P Caso 5



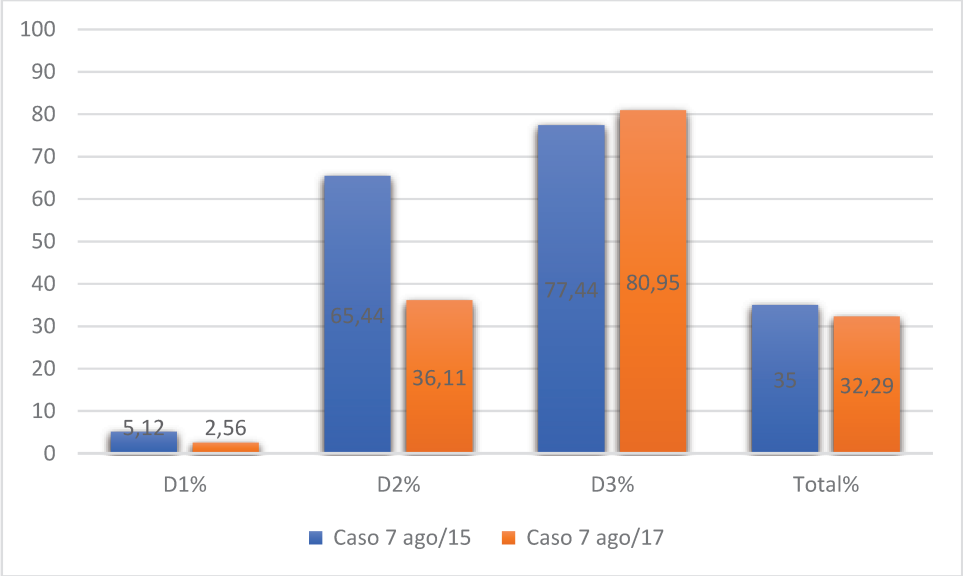
Legenda: D1=dimensão 1; D2 dimensão; D3=dimensão 3; as cores são referents ao ano de avaliação
Eixo y: números em %

Gráfico 6. Evolução MFM-P Caso 6



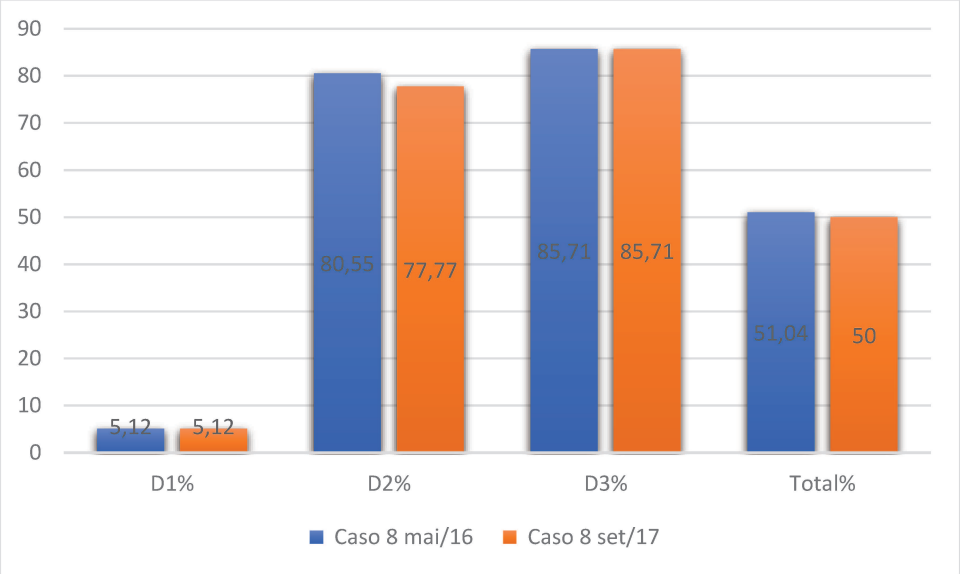
Legenda: D1=dimensão 1; D2 dimensão; D3=dimensão 3; as cores são referents ao ano de avaliação
Eixo y: números em %

Gráfico 7. Evolução MFM-P Caso 7



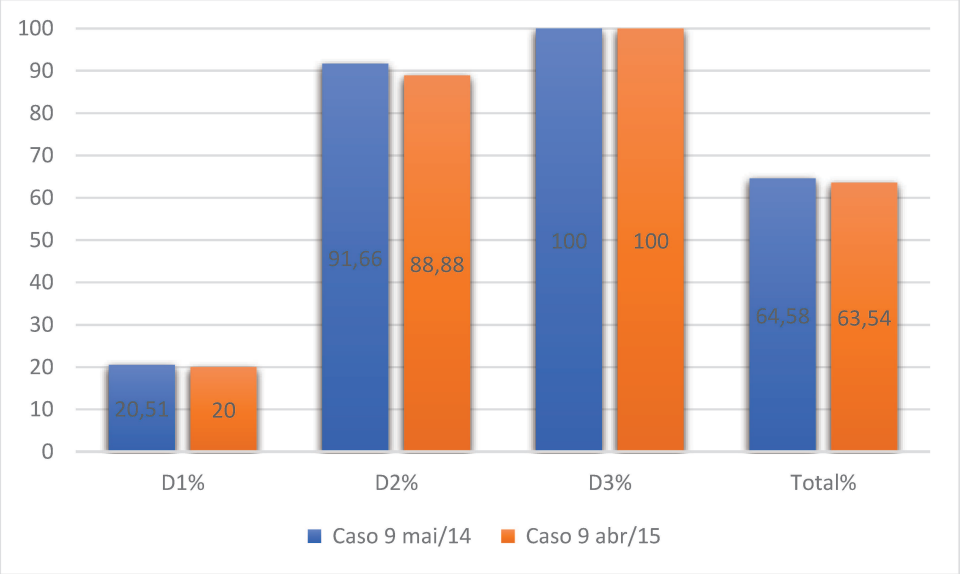
Legenda: D1=dimensão 1; D2 dimensão; D3=dimensão 3; as cores são referents ao ano de avaliação
Eixo y: números em %

Gráfico 8. Evolução MFM-P Caso 8



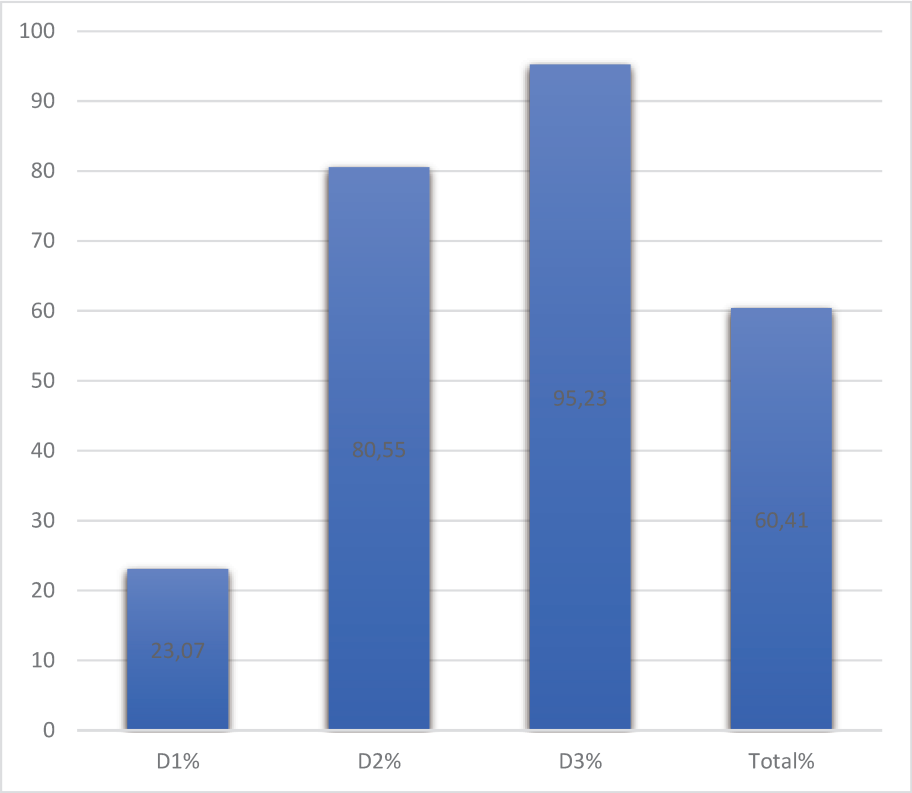
Legenda: D1=dimensão 1; D2 dimensão; D3=dimensão 3; as cores são referents ao ano de avaliação
Eixo y: números em %

Gráfico 9. Evolução MFM-P Caso 9



Legenda: D1=dimensão 1; D2 dimensão; D3=dimensão 3; as cores são referents ao ano
Eixo y: números em %

Gráfico 10. Avaliação MFM-P Caso 10 em outubro/2014

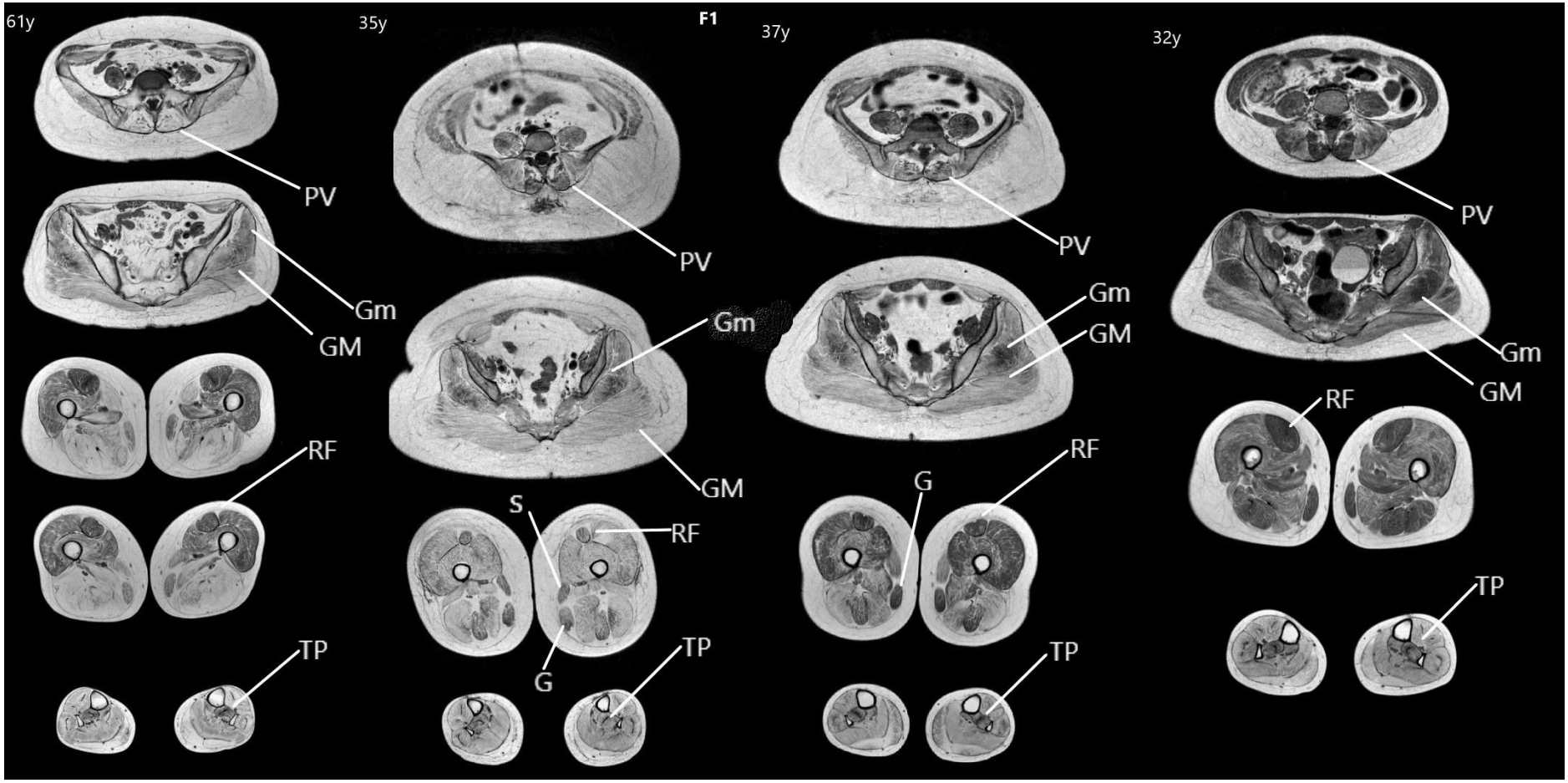


Legenda: D1=dimensão 1; D2 dimensão; D3=dimensão 3; as cores são referents ao ano
Eixo y: números em %

Na figura 1a são apresentadas as imagens de RMm de membros da mesma familia, em relação a região pélvica, coxas e pernas, mostrando comprometimento muscular heterogeneo, porém, em todos casos (F1-1 a F1-4) houve a preservação parcial do músculo tibial posterior e do extensor longo do hálux, quando avaliado através da escala de Mercuri[1](vide Tabelas 3 e 4).

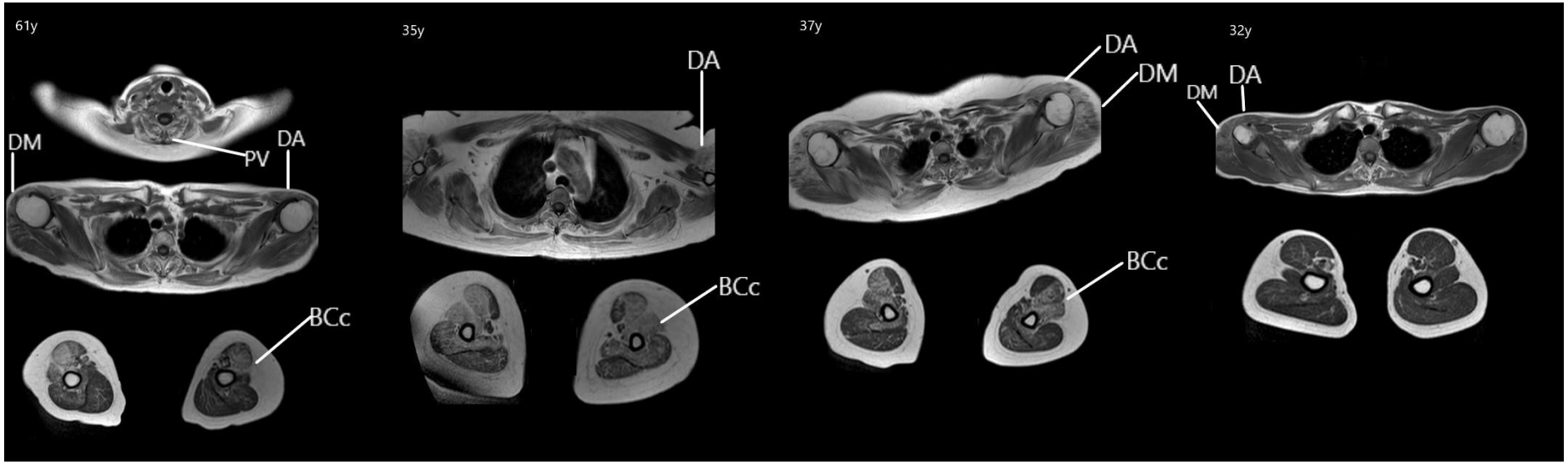
Nas imagens de cintura escapular e braços, para os casos F1-1 a F1-4, foi possível observar que o músculo deltóide anterior, cabeça curta do bíceps braquial e paravertebrais cervicais, apresentavam maior grau de infiltração gordurosa (Figura 1b e Tabelas 3 e 4).

Figura 1a. Imagens de RMm da pelve e membros inferiores dos casos da Família 1 (F-1:casos1-4).



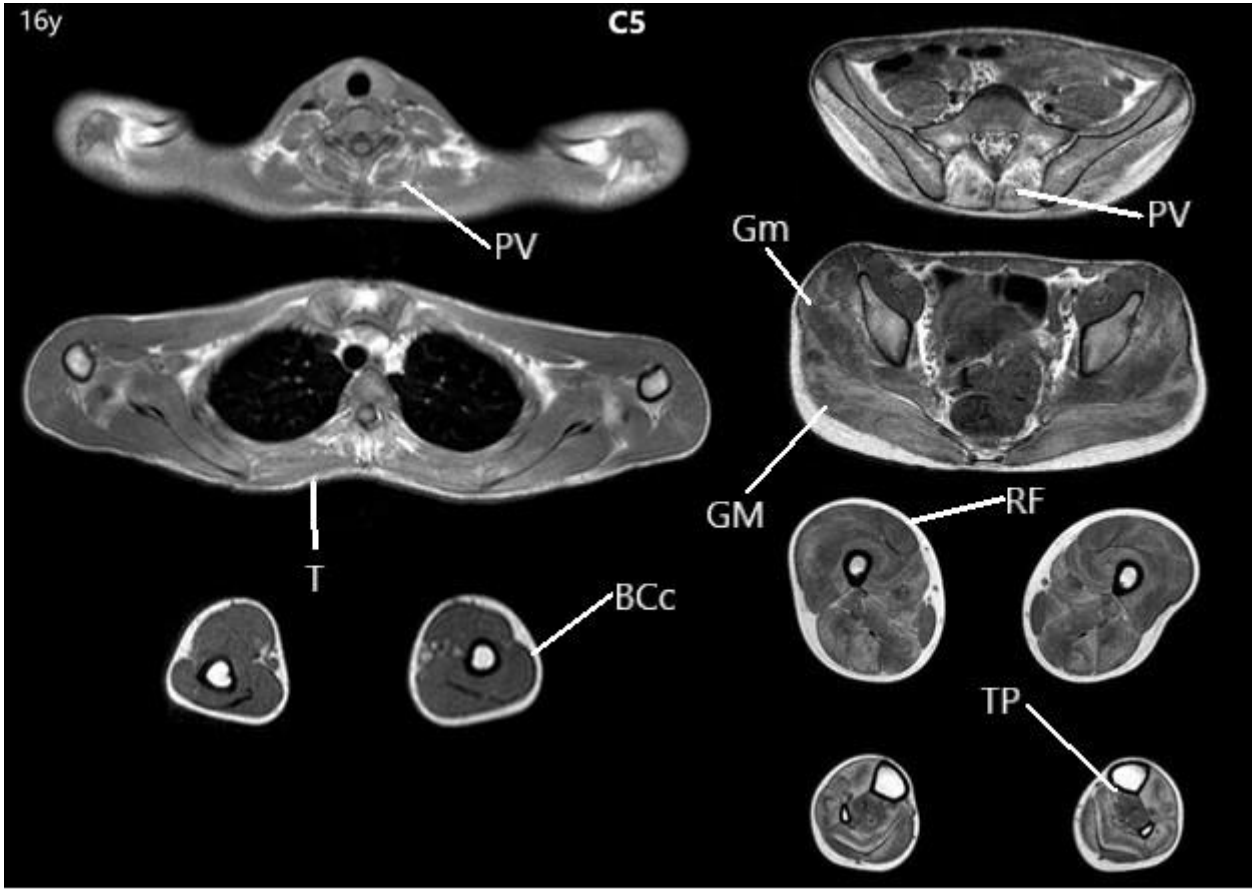
Legenda: F1: família 1; idade em anos (y = year); PV: paravertebrais; RF: reto femoral; GM: glúteo máximo; Gm: glúteo médio; G: grácil; S: sartório TP: Tibial posterior.

Figura 1b: Imagens de RMm cervical, cintura escapular e braços dos casos da Família 1 (F-1: casos1-4).



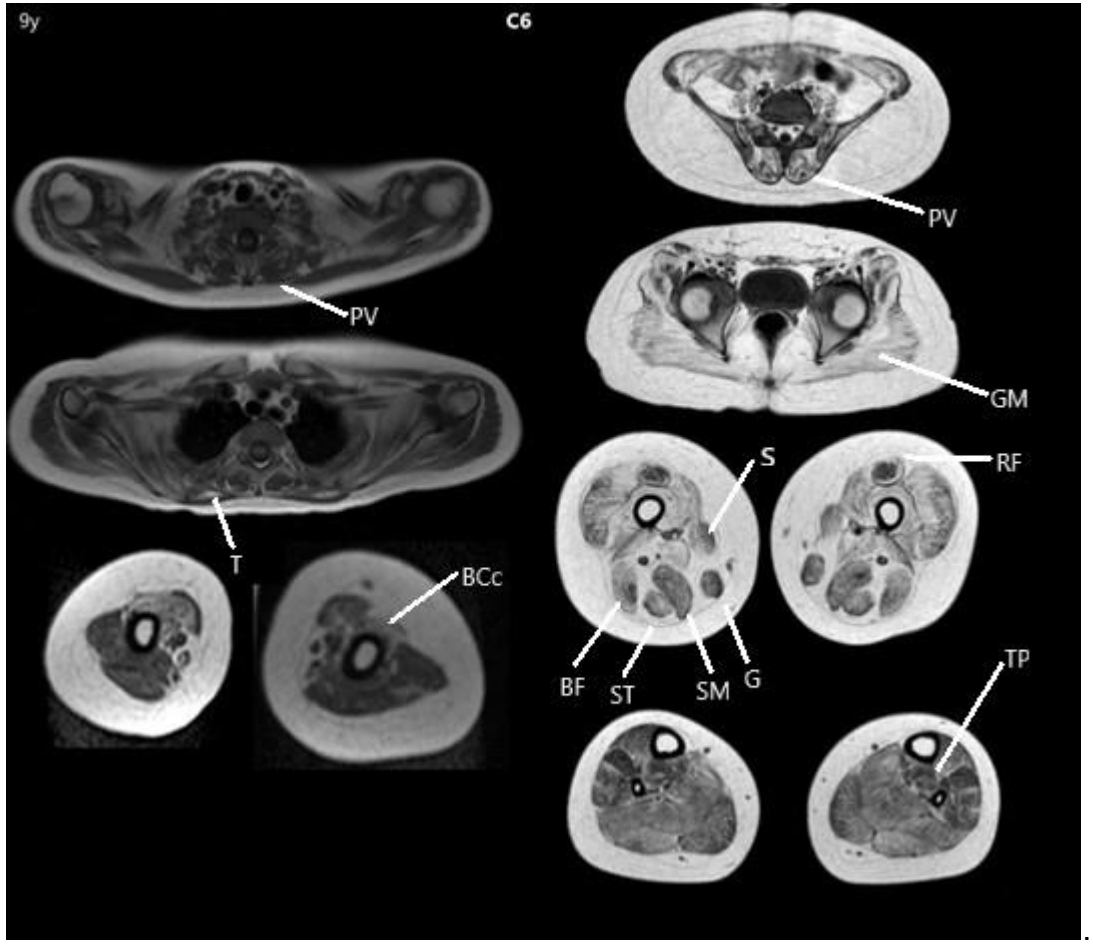
Legenda: F1: família 1; idade em anos (y = year); PV: paravertebrais;DA: deltóide anterior; deltóide médio;BCc: biceps cabeça curta.

Figura 2. Imagens de RMm do Caso 5.



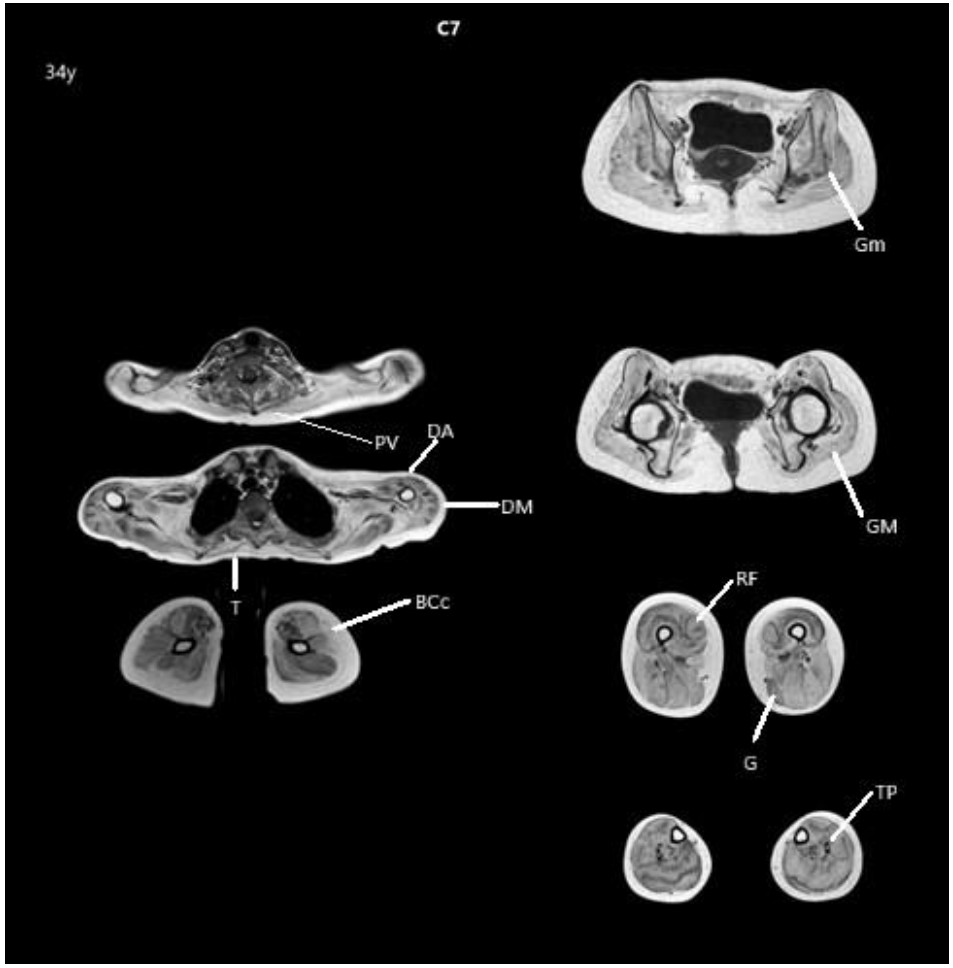
Legenda: C5: caso 5; idade em anos (y = year); PV: paravertebrais; T: trapézio; BCc: biceps cabeça curta; RF: reto femoral; GM: glúteo máximo; Gm: glúteo médio; G: grácil; TP: Tibial posterior.

Figura 3. Imagens de RMm do Caso 6



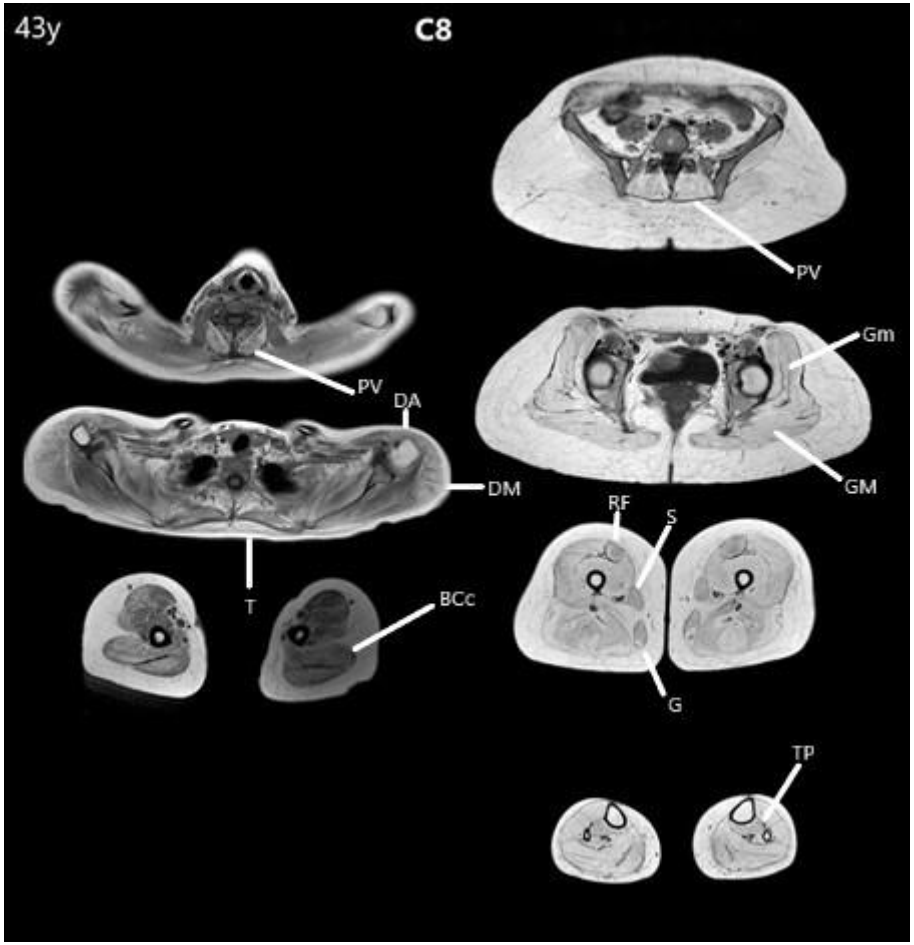
Legenda: C6: caso 6; idade em anos (y = year); PV: paravertebrais; DA: deltóide anterior; deltóide médio; BCc: biceps cabeça curta; T: trapézio; RF: reto femoral; GM: glúteo máximo; Gm: glúteo médio; G: grácil; S: sartório; SM: semimembranoso; ST: semitendinoso; BF: biceps femoral; TP: Tibial posterior.

Figura 5. Imagens de RMm do Caso 7



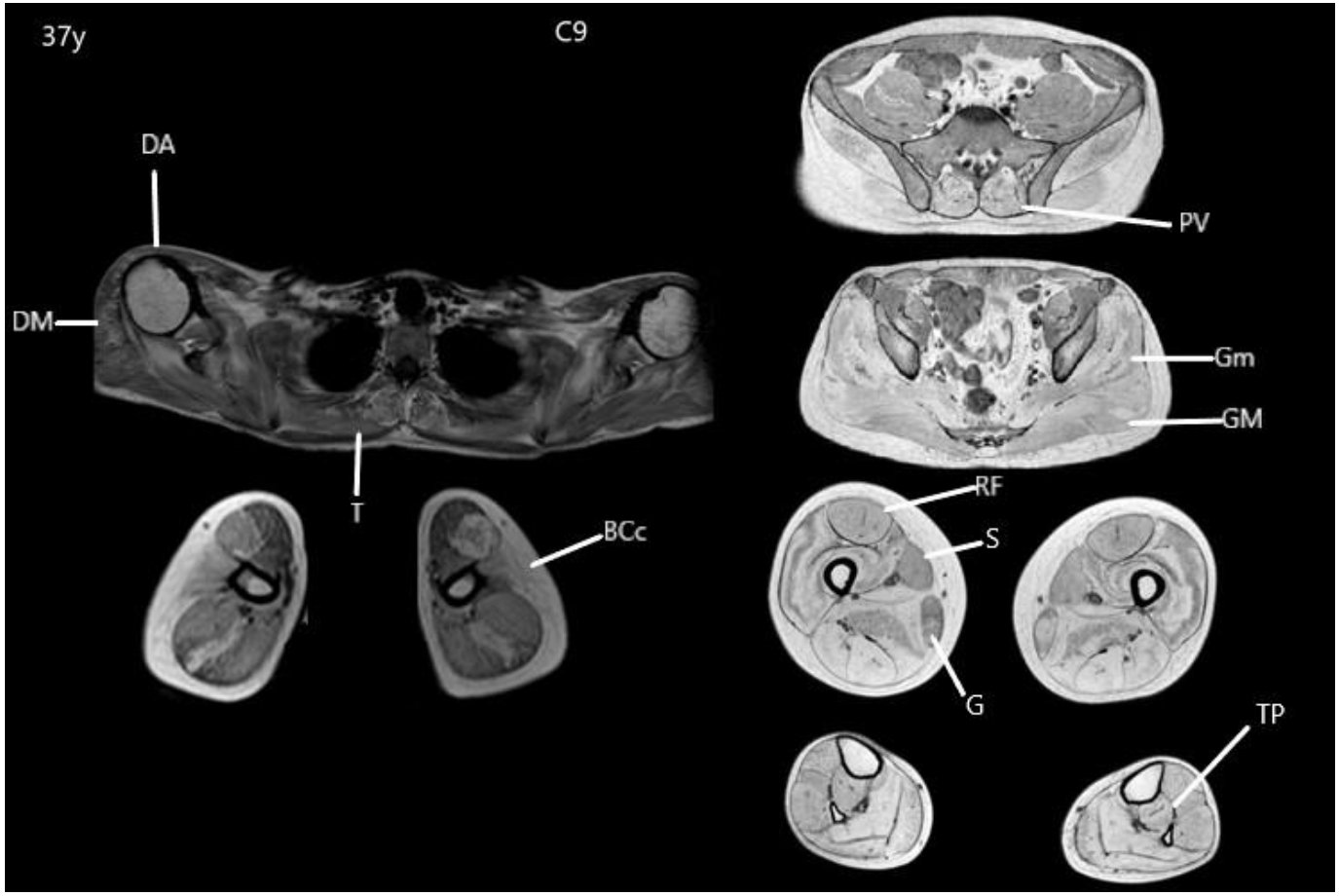
Legenda: C7: caso 7; idade em anos (y = year); PV: paravertebrais; DA: deltóide anterior; deltóide médio; BCc: biceps cabeça curta; T: trapézio; RF: reto femoral; GM: glúteo máximo; Gm: glúteo médio; G: grácil; TP: Tibial posterior.

Figura 6. Imagens de RMm do Caso 8



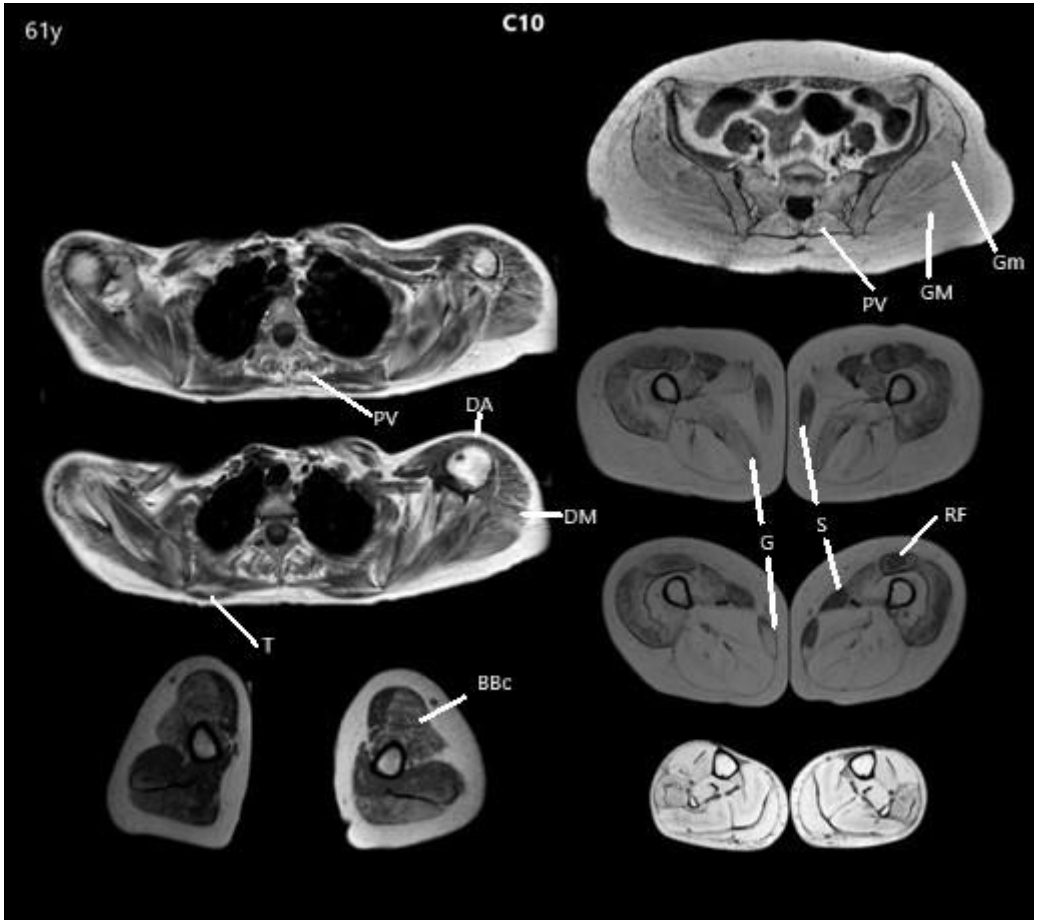
Legenda: C8: caso 8; idade em anos (y = year); PV: paravertebrais; ; DA: deltóide anterior; deltóide médio; BCc: biceps cabeça curta; T: trapézio; RF: reto femoral; S: sartório; GM: glúteo máximo; Gm: glúteo médio; G: grácil; TP: Tibial posterior.

Figura 7. Imagens de RMm do Caso 9



Legenda: C9: caso 9; idade em anos (y = year); PV: paravertebrais; DA: deltóide anterior; deltóide médio; BCc: biceps cabeça curta; T: trapézio; RF: reto femoral; S: sartório; GM: glúteo máximo; Gm: glúteo médio; G: grácil; TP: Tibial posterior.

Figura 8. Imagens de RMm do Caso 10



Legenda: C10: caso 10; idade em anos (y = year); PV: paravertebrais; DA: deltóide anterior; deltóide médio; BCc: biceps cabeça curta; T: trapézio;RF: reto femoral; GM: glúteo máximo; Gm: glúteo médio; G: grácil; TP: Tibial posterior.

As tabelas 3 e 4 apresentam os escores das imagens de músculo desta série de 10 casos, sendo graduado através do proposto por Mercuri et al [5].

Tabela 3. Escores Segundo Mercuri *et al* [1], das imagens de RMm em cintura pélvica, coxas e pernas em 10 pacientes com MCN

Caso	*F1-1*		*F1-2*		*F1-3*		*F1-4*		*5*		*6*		*7*		*8*		*9*		*10*	
Lado	D	E	D	E	D	E	D	E	D	E	D	E	D	E	D	E	D	E	D	E
m.psoas	2	2	3	3	1	1	1	1	2	2	3	3			4	4	3	3	2,5	2,5
m.glúteo máximo	3	3	4	4	3	3	3	3	3	3	4	4	4	4	4	4	4	4	4	4
m.glúteo médio	3	3	3	3	2,5	2,5	2	2	2,5	2,5	4	4	3	3	4	4	4	4	4	4
m.glúteo minimo	4	4	4	4	4	4	4	4	3	3	4	4	4	4	4	4	4	4	4	4
mm. paravertebrais	3	3	2,5(E); 3(L)	2,5(E); 3(L)	3	3	1(E); 2,5(L)	1(E); 2,5(L)	3	3	3	3	3	3	4	4	4	4	4	4
m.reto femoral	2	2	2,5		2,5		1	1	2,5	2,5	2,5	2,5	3	3	4	4	3	3	3	3
m.vasto medial	2,5	2,5	3	3	2	2	3	3	3	3	3	3	4	4	4	4	4	4	4	4
m.vasto intermédio	2,5	2,5	3	3	3	3	3	3	3	3	4	4	4	4	4	4	4	4	4	4
m.vasto lateral	2,5	2,5	3	3	2	2	2	2	3	3	3	3	3	3	4	4	3	3	3	3
m.sartório	3	3	2,5	2,5	3	3	3	3	3	3	3	3	4	4	4	4	3	3	3	3
m.bíceps femoral	4	4	3	3	3	3	2	2	3	3	3	3	4	4	4	4	4	4	4	4
m.semitendinoso	4	4	3	3	2,5		2	2	3	3	3	3	4	4	4	4	4	4	4	4
m.semimembranso	4	4	3	3	3	3	3	3	3	3	3	3	4	4	4	4	4	4	4	4
m. adutor longo	2,5	2,5	3	3	3	3	2	2	3	3	3	3	4	4	4	4	4	4	4	4
m.adutor breve	2,5	2,5	3	3	3	3	2,5	,25	3	3	3	3	4	4	4	4	4	4	4	4
m.adutor maior	2,5	2,5	3	3	3	3	3	3	3	3	3	3	4	4	4	4	3	3	4	4
m.grácil	3	3	2,5	2,5	2,5		1	1	2,5	2,5	2,5	2,5	3	3	4	4	3	3	3	3
m.tíbial anterior	4	4	4	4	4	4	4	4	3	3	3	3	4	4	4	4	4	4	4	4
m.extensor longo hálux	2,5	2,5	2,5	2,5	2,5		2,5	2,5	2,5	2,5	2,5	2,5	4	4	4	4	4	4	4	4
m.tíbial posterior profunco	2,5	2,5	2,5	2,5	2,5		2,5	2,5	2,5	2,5	2,5	2,5	4	4	4	4	4	4	4	4
m.solear	4	4	4	4	4	4	4	4	3	3	3	3	4	4	4	4	4	4	4	4
m.gastrocnêmio medial	4	4	4	4	3	3	4	4	3	3	3	3	4	4	4	4	4	4	4	4
m.gastrocnêmio lateral	4	4	4	4	4	4	4	4	2,5	2,5	3	3	4	4	4	4	4	4	4	4
m .fibulares	4	4	4	4	4	4	4	4	3	3	3	3	4	4	4	4	4	4	3	3

Legenda: F1: familia 1; m: músculo; c: cabeça; E= m. Eretor espinhal; L= m. longuíssimo.

Tabela 4. Escores segundo Mercuri *et al*[1], das imagens de RMm em cintura escapular e braços em 10 pacientes com MCN

Caso	F1-1		F1-2		F1-3		F1-4		5		6		7		8		9		10	
Lado	D	E	D	E	D	E	D	E	D	E	D	E	D	E	D	E	D	E	D	E
m.trápézio	2,5	2,5	3	3	3	3	2	2	2,5	2,5	2,5	2,5	3	3	3	3	2,5	2,5	3	3
m.paravertebral	3	3	3	3	3	3	2	2	3	3	3	3	3	3	4	4	3	3	3	3
m.rombóide	2,5	2,5	3	3	3	3	2	2	2,5	2,5	3	3	3	3	3	3	3	3	2,5	2,5
m.supre-espinhoso	2	2	3	3	2,5	2,5	2	2	2,5	2,5	3	3	3	3	3	3	3	3	2,5	2,5
m.infra-espinhoso	2	2	3	3	3	3	2	2	2,5	2,5	3	3	3	3	3	3	3	3	3	3
m.subescapular	2	2	3	3	3	3	2	2	2,5	2,5	3	3	3	3	3	3	3	3	2,5	3
m.peitoral maior	2,5	2,5	3	3	3	3	2	2	2,5	2,5	3	3	3	3	3	3	3	3	2,5	2,5
m.deltóide (p. anterior)	3	3	3	3	3	3	2,5	2,5	2,5	2,5	3	3	3	3	3	3	3	-	3	3
m.deltóide (p.lateral)	3	3	-	-	3	3	2,5	2,5	2,5	2,5	3	3	3	3	3	3	3	-	3	3
m.deltóide (p.posterior)	3	3	-	-	3	3	2,5	2,5	2,5	2,5	3	3	3	3	3	3	3	-	3	3
m.tríceps braquial	2,5	2,5	2,5	2,5	2,5	2,5	2	2	2,5	2,5	2,5	2,5	3	3	3	3	3	3	2,5	2,5
m.braquial c.longa	2,5	2,5	2,5	2,5	2	2	2	2	2,5	2,5	2,5	2,5	2,5	2,5	3	3	2,5	2,5	2	2
m.braquial c.curta	3	3	3	3	2,5	2,5	2	2	2,5	2,5	3	3	3	3	3	2,5	3	3	2,5	2,5

Legenda: F1: familia 1; m: músculo; c: cabeça; p: porção.

Referências Capítulo 4

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6.0 DISCUSSÃO GERAL

Miopatias estruturais se expressam por sinais e sintomas característicos que podem aparecer desde o período pré-natal até o nascimento e de início mais tardio, diferenciando-se das distrofias congênitas pelo aspecto histopatológico não distrófico [1-3,16-17]. A genética molecular permitiu identificar diferentes genes envolvidos nas miopatias congênitas estruturais, além de ampla variação de anormalidades em um mesmo gene. Sobreposição de fenótipos são comuns entre as miopatias estruturais e entre o mesmo subgrupo de miopatia estrutural.

As miopatias com centralização nuclear, dentre as miopatias estruturais, são raras e constituírem um grupo heterogêneo de doenças, compreendendo formas congênitas, graves e fatais [2,29], casos de moderada gravidade de início na infância ou adolescência e casos mais leves e de início na vida adulta [14,30].

Neste estudo conseguimos verificar variabilidade entre a genética e os fenótipos na série de casos, no total de 15 pacientes. Deles em relação ao início dos sintomas encontramos 9 casos congênitos, 2 de início na idade adulta, 1 na adolescência e os demais em idades variáveis da infância.

Considerando os dados da literatura, a miopatia de início congênito e mais grave refere-se aos casos de MCN ligados ao cromossomo X, os quais são identificados preferentemente como MMT [8,18,29,31]. Em concordância, no estudo, 4 casos (capítulo 2) foram os mais graves, havendo confirmação genética para 2 deles (casos 3 e 4). A gravidade foi expressa pela morte precoce em um caso e, pela necessidade de ventilação assistida, gastrostomia e prolongado período de internação em unidade de terapia intensiva, em todos eles. Nos pacientes em continuo seguimento temos podido constatar a melhora lenta e progressiva quanto as aquisições motoras, como descrito no capítulo 2, embora com apoio profissional multidisciplinar intensivos.

Ressaltamos que a partir de dados clínicos, a saber, criptorquidia, leve hipospadia e micropênis, associados a dados laboratoriais de hipofunção endócrina, foi possível suspeitar de síndrome de genes contíguos, no caso 3, circunstâncias já descritos na literatura [32-34]. Nesse caso, através de sequenciamento de nova geração, foi documentada a síndrome de genes

contíguos, havendo uma grande deleção (500 mil bases) incluindo o gene *MTM1* e parcialmente o gene *MAMLD1*.

No caso 4 (capitulo 2), o fenótipo clássico para MMT-LX [3,17] , nova variante (c:482_485 del TGGA) no gene *MTM1* foi descoberta. Essa foi considerada patogênica por levar à substituição de triptofano em posição 164 por glutamina, criando um códon de parada prematura. A citada mutação não foi descrita em grandes bancos genômicos até o momento [35].

Ainda em relação ao capitulo 2, merece destaque a avaliação da musculatura (caso 3), através de RMm, descrição inédita na literatura em casos congênitos e em idade precoce. Entretanto, autores [36-37] relataram dados de RMm para casos tardios e em pacientes do sexo feminino, as quais eram casos isolados de mutação *MTM1*, como discutido no artigo.

O capitulo 3 relata uma paciente com MCN, de início congênito, a qual foi erroneamente diagnosticada como distrofia miotônica de Steinert, com base apenas no fenótipo e em exame eletroneuromiografico, realizado na idade adulta, o qual mostrava descargas do tipo miotônicas. De fato, descargas miotônicas ou descargas repetitivas complexas são frequentemente encontradas na doença de Steinert, porém outras doenças mais raras também as apresentam, como tem sido descrito na MCN [38-39]. Como lição do caso, aspectos clínicos e eletromiográficos podem nortear o *work up* diagnóstico, entretanto nunca o definir. Outro detalhe a ser destacado é o fato de que as descargas miotônicas elétricas não eram acompanhadas do fenômeno miotônico clínico. A avaliação da paciente, através da escala MFM-P, mostrou grave comprometimento funcional, pontuando com escore total menor que 70% e para a dimensão 1 (em pé e transferências) escore <40%. A paciente deambulava pequenas distâncias, necessitando de cadeira de rodas para longas distâncias. Entretanto, em pacientes com distrofia muscular de Duchenne, os valores citados eram preditivos para perda da deambulação em um prazo máximo de um ano [40], não havendo relatos na literatura de MFM para pacientes com MCN.

A RMm mostrava padrão de infiltração gordurosa na musculatura pélvica e membros inferiores compatível com o descrito na literatura para casos comprovados de MCN-*DNM2* [41]. O relato do caso contribui para o conhecimento

dos achados de RMm em nível cervical, cintura scapular e braços, ou seja: a) infiltração gordurosa em músculos paravertebrais cervicais; b) cintura scapular menos afetada que cintura pélvica; c) deltóide porção anterior e porção lateral mais afetados que os demais músculos escapulares; d) bíceps, cabeça curta mais compremetido que os demais músculos do braço. Tais dados são superponíveis aos encontrados nos pacientes descritos no capitulo 4, a ser discutido mais abaixo.

A biópsia muscular da paciente previa MCN com mutação no gene *BIN1,* devido a peculiaridades histopatológicas idênticas aos casos com confirmação genética para MCN-*BIN1*[1,42-44]. As citadas peculiaridades incluíam várias fibras musculares com agrupamento nuclear localizado centralmente e um grande número de miofibras com apenas um núcleo central além de atrofia de fibra tipo 1 e hipertrofia das de tipo 2 e poucas fibras com aspecto de “roda de carroça” [1,42-44]. Surpreendentemente a genética molecular mostrou mutação, já descrita, no gene *DNM2*, validando o padrão de imagem de RMm que foi encontrado.

No capítulo 4 estudamos 10 pacientes: uma família (F1:casos 1-4) com herança AD, um (caso 5) com confirmação genética para mutação, já descrita, no gene *DNM2,* um caso da forma AD por história e 4 casos esporádicos. Os 10 pacientes tiveram avaliação funcional pela escala MFM-P (32 itens) e imagem de músculo com padrões descritos na literatura relacionados a mutação do gene *DNM2*[1,3].

Vale ressaltar também a presença de 2 casos (casos 6 e 9) com sobreposição de fenótipos, os quais apresentavam flutuação dos sintomas motores com estimulação repetitiva positiva para alteração em placa motora, que estão sendo estudados com refinamento neurofisiológico em outro projeto de pesquisa. As avaliações da MFM-P apresentadas nesses 2 casos foram realizadas sem o efeito da anticolinesterásicos.

Susman et al. [44] relataram alguns de seus pacientes MCN-*DNM2* com ligeira redução da velocidade de condução nervosa nos membros inferiores. Mori-Yoshimura et al. [45], não encontraram qualquer dessa anormalidade, sugerindo que o envolvimento do nervo periférico não ocorreria com frequência em pacientes MCN-*DNM2*. No presente estudo, a revisão da eletromiografia

mostrou-se anormal em 9 casos, dos quais 8 com características de processo miopático, como é frequentemente observado [14,38-39] e um com alteração tipo neurogênica, também encontrada por Zanoteli et al.[14]. Em nenhum paciente do estudo houve alteração da velocidade de condução, fato que ocorre na neuropatia de Charcot-Marie-Tooth tipo 2B que é causada também por mutação no gene DMN2 [47].

Nenhum dos pacientes, avaliados através da escala MFM, pontuou com escore máximo possível, ou seja, função motora plena, com 100%. A totalidade da casuística mostrou pior desempenho na dimensão D1, que é referente à posição em pé e transferência. Nota-se que a D1 inclui testes que necessitam de função dos músculos de cintura pélvica e escapular, além dos músculos distais de membros inferiores, sendo justamente aqueles músculos mais afetados visto através das imagens de músculo. Os melhores resultados, no geral, foram obtidos em D2 (função axial e proximal) e D3 (função distal), entretanto os pacientes mais graves eram os que tiveram início precoce da doença e, quando avaliados pela RMm mostravam importantes alterações nos músculos da cintura pélvica e escapular e principalmente da musculatura paravertebral.

Os melhores desempenhos na MFM-P estão em D3, como por exemplo juntar moedas em uma das mãos, em um tempo total máximo de 20 segundos. Observa-se que para essa atividade os músculos necessários não apresentam, nestes pacientes, maiores comprometimentos quando comparada aos demais músculos. Considere-se que não temos parâmetros na literatura sobre a MFM na MCN e imagem de músculo através de RMm, para comparação, porém Mul et al.[47] mostrou que quanto maior fração média de gordura em membros inferiores, menor pontuação total para a MFM, em pacientes com distrofia face-escápulo-umeral.

Estudos utilizando a escala MFM, em pacientes com Distrofia Muscular de Duchenne, mostraram que um escore total de 70% e uma pontuação de 40% de D1 são valores que podem prever a perda de deambulação em um prazo de um ano [45]. A menor pontuação total na MFM-P na coorte foi de 32,29% em uma paciente cadeirante (caso 7), também no caso 8 a regra acima citada [45] foi respeita. Os pacientes deambulantes apresentaram MFM-P ≥68,75%. Entretanto, a marcha era possível com o uso de dispositivo auxiliar em dois de

nossos casos, um (caso F1-1) com escore total de 62,5% e 28,2% em D1 e outro (caso 9) com escore total de 64,58% e 20,51% na D1 da escala MFM.

No estudo descrevemos normalidade ou alterações pela RMm nas regiões cervicais, de cinturas escapular e pélvica, braços, coxas e pernas, enquanto na literatura as informações sobre imagens de músculo na MCN-*DNM2* referem-se à cintura pélvica e membros inferiores [3, 7, 45,46].

Na coorte a cintura escapular, como um todo, foi menos afetada pela miopatia em relação a cintura pélvica, entretanto o deltoide (porção anterior e lateral) foi o que mais expressou alteração do sinal em imagens ponderadas em T1w. No braço, a cabeça curta do bíceps braquial mostrou-se mais afetada. Na cintura pélvica os músculos glúteos máximo e mínimo são mais afetados que o glúteo médio, embora nos casos mais graves todo o grupo está igualmente afetado. Nas coxas os músculos reto femoral, grácil e sartório foram os menos comprometidos. Nas pernas os músculos tibiais posterior e extensor longo do hálux apresentam menor intensidade de sinal expressando menor infiltração gordurosa mesmo nos casos mais graves. A musculatura paravertebral tanto cervical, quanto lombar mostram-se anormais sugerindo que a miopatia tem componente axial. No total, a RMm pode ser considerada como um biomarcador da gravidade da MCN.

Durante a realização do estudo foi coletada amostra de sangue para a realização da genética molecular de todos os pacientes relatados no capítulo 4, não sendo possível a confirmação genética para todos, deixando-se a execução dos testes para futuro próximo, assim como a publicação dos dados referentes ao citado capítulo.

7.0 CONCLUSÃO

O estudo permitiu:

Descrição de nova mutação causal no gene da Miotubularina.

Descrição clínica, de imagem por RMm e genética de um caso com a síndrome de genes contíguos (*MTM1/MAMLD1*).

Expansão do conhecimento morfológico na miopatia centronuclear por mutação no gene da Dinamina 2.

Constatar que a avalição funcional pela MFM-P com score total ≥68,75% se associou a pacientes MCN deambulantes.

Demonstrar que a RMm teve padrão de alteração do sinal correspondente a infiltração gordurosa maior em pernas-pélvis e menor em cintura escapular. Os músculos reto femoral, grácil e sartório foram relativamente preservados. Nas pernas, os músculos tibial posterior e extenso longo do hálux foram os menos afetados, em concordância com a literatura em casos de MCN-*DNM2*. A cabeça curta do músculo bíceps braquial, músculos deltoides anterior e lateral e musculatura paravertenral cervical tiveram infiltração gordurosa, acrescentando conhecimentos sobre a RMm na MCN.

8.0 REFERÊNCIAS GERAIS

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ANEXOS

ANEXO 1

Aprovação Comitê de Ética

FACULDADE DE CIENCIAS MEDICAS - UNICAMP (CAMPUS CAMPINAS)

Plataforma Brasil

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: MIOPATIAS COM CENTRALIZAÇÃO NUCLEAR

Pesquisador: TATIANA DA SILVA ROSA

Área Temática: Genética Humana:
(Trata-se de pesquisa envolvendo Genética Humana que não necessita de análise ética por parte da CONEP;);

Versão: 6

CAAE: 26988114.1.0000.5404

Instituição Proponente: Hospital de Clínicas da UNICAMP

Patrocinador Principal: Universidade Estadual de Campinas - UNICAMP

DADOS DO PARECER

Número do Parecer: 834.006

Data da Relatoria: 09/10/2014

Recomendações:

A Res. CNS 196-96 foi revogada e substituída pela Res. CNS 466-2012. Corrigir os documentos com a informação correta.

Conclusões ou Pendências e Lista de Inadequações:

Projeto aprovado e liberado para coleta de dados, considerando a resolução satisfatória de pendências emitidas em pareceres anteriores, após solicitação de recurso pelos pesquisadores.

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

Considerações Finais a critério do CEP:

- Se o TCLE tiver mais de uma página, o sujeito de pesquisa ou seu representante, quando for o caso, e o pesquisador responsável deverão rubricar todas as folhas desse documento, apondo suas assinaturas na última página do referido termo (Carta Circular nº 003/2011/CONEP/CNS).

Endereço: Rua Tessália Vieira de Camargo, 126
Bairro: Barão Geraldo **CEP:** 13.083-887
UF: SP **Município:** CAMPINAS
Telefone: (19)3521-8936 **Fax:** (19)3521-7187 **E-mail:** cep@fcm.unicamp.br

FACULDADE DE CIENCIAS MEDICAS - UNICAMP (CAMPUS CAMPINAS)

- Cabe ao pesquisador desenvolver a pesquisa conforme delineada no protocolo aprovado, elaborar e apresentar os relatórios parciais e final, bem como encaminhar os resultados para publicação com os devidos créditos aos pesquisadores associados e ao pessoal técnico participante do projeto (Resolução 466/2012 CNS/MS). Os relatórios deverão ser enviados através da Plataforma Brasil- ícone Notificação.
- Eventuais modificações ou emendas ao protocolo deverão ser apresentadas ao CEP de forma clara e sucinta, identificando a parte do protocolo a ser modificada (com destaque) e suas justificativas. As modificações deverão ter parecer de aprovação prévia deste CEP.

CAMPINAS, 16 de Outubro de 2014

Assinado por:
Renata Maria dos Santos Celeghini
(Coordenador)

ANEXO 2

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO (TCLE)

Pesquisa: Miopatias com centralização nuclear.

Prezado (a) Senhor (a):

O(a) senhor(a) ou o(a) menor do qual é o representante legal está sendo convidado(a) a participar da pesquisa, porque já tem o diagnóstico de Miopatia centronuclear ou miotubular (miopatias com centralização nuclear).

Favor ler estas folhas cuidadosamente. Elas explicarão sobre o estudo e ajudarão a decidir participar ou não.

1- O estudo tem como objetivo conhecer aspectos clínicos, de imagem e genéticos das miopatias com centralização nuclear, para melhor caracterizá-las.

2- Propõe ainda trazer conhecimentos sobre essas miopatias, o que poderá ajudar no seu manuseio clínico, permitir aconselhamento genético e orientar quanto ao prognóstico e nas adaptações nas atividades laborais e de vida diária.

PROCEDIMENTOS, RISCO E DESCONFORTO:

3- Neste estudo serão realizadas avaliações por neurologista, outros médicos e fisioterapeutas, abordando vários aspectos da sua doença anteriormente já diagnosticada no Ambulatório de Doenças Neuromusculares, com os nomes de miopatia miotubular ou centronuclear, quando no passado, se submeteu a uma biópsia de músculo.

4 - Os testes programados para a realização da pesquisa incluem:

4a.) exame clínico neurológico (semelhantes às consultas rotineiras, nas quais a força é testada e a escala motora aplicada) com duração aproximada de 25 minutos. O desconforto previsível nesta situação poderá ser mínimo.

4b.) testes pulmonares (inspirar e expirar em um aparelho), com duração aproximada de 15 minutos. Neles poderá haver algum desconforto, em geral leve, quando sopra no bocal do aparelho.

4c.) exame de ressonância magnética para avaliar músculos e cérebro com duração aproximada de 45 minutos. Não haverá riscos, considerando que não será injetada qualquer substancia no paciente. Antes do exame um questionário é respondido pelo paciente para excluir as contraindicações do exame (peças de metal no corpo, pânico de locais fechados, por exemplos). Pode haver desconforto pela necessidade de manter-se imóvel, entretanto haverá comunicação através de radio, entre o paciente e o médico, e serão permitidas pausas. Também poderá haver a interrupção do exame caso o paciente assim o deseje.

4d) teste psicométrico (questionário com perguntas simples), realizado por um psicólogo com tempo aproximado de 15 minutos. Não há riscos, porém pode haver algum desconforto.

4e.) biofotometria (medidas nas articulações, através da colocação de etiquetas em pontos do corpo e a realização de fotos). A identidade pessoal de todos os pacientes será preservada, através da utilização de máscara e gorro cirúrgicos, os quais impossibilitam a identificação. As imagens serão utilizadas para fins acadêmicos, incluída sua publicação na literatura científica especializada. Nesse procedimento não há risco e o desconforto pode ser mínimo e será realizado no tempo de aproximadamente 20 minutos.

4f.) teste genético para avaliação de mutações. Para isso uma coleta de 20 a 30 ml de sangue de uma veia do braço, que será realizada por profissional treinado para esse procedimento. Entretanto, pode haver desconforto e possíveis riscos, como dor e manchas roxas (equimoses) no local da coleta do sangue.

Os procedimentos acima citados serão realizados durante as consultas programadas no Ambulatório de Neuromuscular-UNICAMP, como ocorre regularmente 03 vezes ao ano. Para a realização da Ressonância haverá único agendamento específico, ocasião que será oferecido o ressarcimento do valor gasto somente com transporte.

BENEFÍCIOS:

Os pacientes não irão obter qualquer benefício direto e imediato com a sua participação nesse estudo e o seu diagnóstico e o seu tratamento provavelmente não serão modificados. Contudo, os resultados desse estudo podem, em longo prazo, oferecer vantagens para os indivíduos com doenças neuromusculares e suas famílias, possibilitando um melhor diagnóstico para manuseios mais adequados.

Será garantida a todos os pacientes a apresentação dos resultados obtidos em todas as avalições realizadas durante o estudo e ainda o aconselhamento

genético, esse sendo oferecido no Ambulatório de Neurogenética do Hospital de Clínicas (HC) da Universidade Estadual de Campinas (UNICAMP).

SIGILO:

Todas as informações resultantes da pesquisa farão parte do prontuário médico e serão submetidas aos regulamentos do HC-UNICAMP referentes ao sigilo da informação médica. Os resultados serão usados em publicação científica, entretanto, sem revelar a identidade do paciente.

O (a) senhor (a) esta livre para interromper a qualquer momento sua participação na pesquisa. A sua interrupção não causará prejuízo ao seu atendimento, cuidado e tratamento no Hospital da UNICAMP.

FORNECIMENTO DE INFORMAÇÃO ADICIONAL:

A qualquer momento o (a) senhor (a) poderá requisitar informações adicionais relativas ao estudo. A fisioterapeuta Tatiana da Silva Rosa, tel (19) 993358618 e-mail tatirosas@gmail.com e a Profª Drª Anamarli Nucci, tel (19) 3521-7273, estarão disponíveis para responder suas questões e preocupações relacionadas à pesquisa nos respectivos telefones ou ainda no Ambulatório de Doenças Neuromusculares do Hospital de Clínicas da UNICAMP, todas as terças-feiras de 8h as 12h, sendo a pesquisa de total responsabilidade das pesquisadoras.

Em caso de denúncias e/ou reclamações referente somente aos aspectos éticos da pesquisa o (a) senhor (a) poderá contatar a secretaria do Comitê de Ética em Pesquisa, no seguinte endereço: Rua Tessália Vieira de Camargo, 126 Barão Geraldo CEP: 13.083-887 Campinas - SP tel. (19) 3521-8936 Fax: (19)3521-7187 e-mail: cep@fcm.unicamp.br

Declaro que li e entendi esse documento, estou ciente e concordo com os esclarecimentos prestados.

Rubrica do pesquisador	Rubrica do sujeito de pesquisa ou seu representante legal.

Eu confirmo que o(a) FT(a)._____
me explicou o objetivo do estudo, os procedimentos aos quais serei submetido, os riscos, os desconforto e as possíveis vantagens advindas desse projeto de pesquisa. Eu li e compreendi (ou me foi explicado) esse termo de consentimento e estou de pleno acordo em participar desse estudo.

Além disso, informo que em relação ao teste genético para avaliação de mutações:
( ) Autorizo o armazenamento do material biológico e dispenso a necessidade de novo consentimento em caso de seu uso em outras pesquisas.
( ) Autorizo o armazenamento do material biológico e desejo ser consultado para consentimento em caso de seu uso em outras pesquisas.
( ) **NÃO** autorizo o armazenamento do material biológico, devendo o mesmo ser descartado após o encerramento de minha participação nessa pesquisa.

Em relação às imagens que serão obtidas durante as avaliações:
( ) Autorizo a publicação de minha imagem, com preservação da identidade
( ) **NÃO** autorizo a publicação de minha imagem, com preservação da identidade

_____
Assinatura do participante ou representante:

_____
Nome do participante ou representante legal.

_____
Assinatura do participante ou representante legal. Data: ___/____/_____.

_____
Nome da testemunha

_____

Assinatura da testemunha Data: ___/____/_____.

RESPONSABILIDADE DO PESQUISADOR:

Eu expliquei a _____, o objetivo do estudo, os procedimentos requeridos e os possíveis riscos e vantagens que poderão advir do estudo, usando o melhor do meu conhecimento. Eu me comprometo a fornecer uma segunda via original desse termo de consentimento ao participante ou responsável. Caso uma nova pesquisa seja realizada utilizando o material biológico coletado e armazenado por ocasião dessa pesquisa, comprometo-me a submeter e aguardar o parecer do sistema CEP/CONEP para sua utilização.

_____

Nome do pesquisador ou associado.

_____

Assinatura do pesquisador ou associado. Data: ___/____/_____.

Nome do paciente: _____

RG: _____ HC: _____

Endereço: _____

Telefone de contato: _____

Obs.: Este TCLE será assinado em duas vias originais, sendo uma delas destinada ao participante da pesquisa.