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Founders: Giovanni Nigro and Lucia Ines Comi

Three-monthly

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EDITORIAL

New graphic for Acta Myologica

Acta Myologica is the oldest international myology journal, first published in June 1982 with the name of Cardiomyology. At present, it is the official journal of the AIM (Associazione Italiana di Miologia) and of the MSM (Mediterranean Society of Myology).

Starting from October 2016, Acta Myologica has gone online with an open web-based access, with the purpose to renew this historical printed journal devoted to clinical and molecular myology by spreading new knowledge through electronic media platforms.

Acta Myologica Online (AMO) publishes readily accessible research of clear relevance for diagnosis and treatment of muscle disorders, with a special focus on neuromuscular disorders and cardiomyopathies, adapting its graphics into an indexed journal. The changes are available in the Authors Instructions.

As established at the latest meeting of the Board, from the current year, the Authors will partially cover the costs of publication, with a contribution of Euros 200. To the Authors members of the AIM or MSM Scientific Societies (current AIM or MSM membership required) a 50% off is offered.

Luisa Politano
Editor in Chief
Acta Myologica

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ORIGINAL ARTICLES

Coagulation disorders in Duchenne muscular dystrophy? Results of a registry-based online survey

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Different complications of hemostasis have been reported in patients with Duchenne Muscular Dystrophy (DMD). These comprise an increased rate of bleeding-symptoms during scoliosis surgery but also thromboembolic complications such as pulmonary embolism, cerebral infarction, deep vein thrombosis or cardiac thrombus.

For this cross-sectional study, personalized online survey-links were forwarded to 682 registered patients with a genetically confirmed diagnosis of DMD via the German-Austrian DMD patient registry (www.dmd-register.de). The questionnaire enquired data regarding the degree of mobility, disposition to hematoma, epistaxis and gum bleeding, occurrence of peri- and postsurgical hemorrhage, stroke, deep vein thrombosis, and cardiac thromboembolism. Further data on regular medication and age were recorded.

Three-hundred-fifty-one DMD-patients completed the questionnaire (response rate of 51.5%). Of those, 164 (46.7%) were ambulatory and 187 (53.3%) were non-ambulatory. Age distribution was homogeneous. Two participants had a history of thromboembolic events (0.6%). Correlations analysis revealed no coherence with the degree of mobility, age or regular medication. A bleeding tendency was reported by 76 participants (21.7%). No significant correlations with age or degree of mobility were found. We found no association with underlying genetic variants. Results of this patient registry-based survey do not indicate a distinct DMD-specific risk for thromboembolic events that exceeds the risk by typical comorbidities of chronic immobility and cardiac insufficiency in advanced stages of the disease. The results of this survey suggest a mild bleeding tendency in this DMD cohort, whereas a selection bias cannot be excluded.

Key words: Duchenne muscular dystrophy, coagulopathy, bleeding tendency

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Conflict of interest
The Authors declare no conflict of interest

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Introduction

Duchenne muscular dystrophy (DMD) is a rare genetic disease leading to chronic and progressive degeneration of muscle tissue. First symptoms of muscle weakness typically occur in pre-school age. With further

progression, loss of ambulation occurs in teenage years, followed by development of scoliosis, respiratory insufficiency, and dilated cardiomyopathy. DMD is caused by mutations in the dystrophin gene, which lead to a loss of function of the dystrophin protein [1]. Along with beta-dystroglycan, dystrobrevin and syntrophin, dystrophin is part of the dystrophin-associated protein complex (DAPC), connecting extracellular matrix to muscular cytoskeleton. While full-size dystrophin (427kDa) is expressed predominantly in skeletal muscle cells and myocardial cells, smaller isoforms are also expressed in other tissues. In most cases, only male individuals are affected by DMD as mutations in dystrophin follow an X-linked pattern of inheritance. Prevalence of the disease was estimated to be about 4.8 in 100,000 male individuals worldwide, while incidence ranges between 15.9 to 19.5 per 100,000 live born males per year [2].

In patients with DMD both an increased risk of bleeding as well as thromboembolic events have been discussed. Especially bleeding-complications during scoliosis surgery in DMD have been described repeatedly [3,4]. Several case-reports or smaller retrospective studies reported pulmonary embolism [5], cerebral infarction [6-9], deep vein thrombosis [10] or cardiac thrombus [11]. The incidence of cerebral infarction in patients with DMD has been estimated to be around 0.75-1.8% and is thereby notably higher than in the general population [8]. As the smaller dystrophin isoform dp71 is also expressed in platelets, a disease-specific disorder of thrombocytic function and hence in primary hemostasis in DMD appears possible [12-14]. The aim of this cross-sectional survey was to explore if disease-specific complications due to undetected coagulation disorders are present in patients with DMD and eventually depend on age and degree of mobility.

Methods

We used a two-step approach with an initial screening questionnaire and specific follow-up questions. The screening questionnaire consisted of 9 questions assessing (1) age, the degree of mobility and long-term medication, (2) bleeding tendency (disposition to hematoma, epistaxis or gum bleeding and occurrence of peri- or postsurgical hemorrhage) and (3) thromboembolic events in the past (stroke, deep vein thrombosis, cardiac thromboembolism). During a pilot phase, neuromuscular and hemostaseologic specialists from the University of Freiburg reviewed, tested and optimized the questionnaire (for complete questionnaire see supplemental Table II). Inclusion criteria of this study were (1) registration in the German-Austrian DMD patient-registry (*www.duchenne-register.de*, based at the Friedrich-Baur-Institute, Ludwig-Maximilians-University of Munich, Germany), which involves deposition of genetic confirmation and (2) present residence in Germany. No exclusion criterion was defined. No personal data from the patient registry were forwarded to the study center. The corresponding Ethics Committee and the oversight committee of the DMD patient-registry approved the project. For distribution of the questionnaire, we used the online platform "SurveyMonkey.com" and generated personalized links. Registry curators sent these links by e-mail or surface mail to each registered patient. The link also provided the option to decline participation. In case of no answer we sent two reminders. Double use of individual online-questionnaires was traceable. Patients were offered to provide their consent and contact details for further follow-up questions.

Patients giving consent for follow up were contacted differently: In case of a reported bleeding tendency, patients received a more precise questionnaire based on the answers provided in the initial survey. If perioperative or postoperative hemorrhages in the past had been indicated in the initial survey, patients were contacted by phone and corresponding medical reports were requested.

To analyze whether disorders of coagulation are associated with the type of genetic mutations, the underlying genetic findings of all participants were assessed and grouped in large mutations (deletions or duplications of 1 exon or larger), small mutations (deletions or insertions < 1 exon, splice site mutations, point mutations), and intronic mutations according to previous studies [15]. Mutations downstream of exon 63 are known to disrupt the expression of the shortest dystrophin isoform dp71 [16], so that mutations were further grouped by localization within the dystrophin gene (upstream of exon 30; exons 31 to 62; downstream of exon 63).

We analyzed clinical data descriptively and processed them with absolute frequencies and percentage values. For statistical analysis we used SPSS (version 22.0) and performed correlation analysis using a two-sided approach for ordinal scaled parameters (Kendall-Tau-b).

Results

The survey was conducted between October 2017 and January 2018. In October 2017, 1459 patients were registered in the DMD patient-registry. Of those 682 fulfilled the inclusion criteria and were included in this study (see Figure 1 for a flowchart of the study). A total of 351 DMD-patients/caregivers completed the questionnaire (response rate of 51.5%). Age distribution was homogeneous (< 10 years = 36.1%; 11-15 years = 20.5%; > 15 years = 39.4%). Of all participants 164 (46.7%) were ambulatory and 187 (53.3%) were non-ambulatory. Regular medication was taken by 259 (73.8%) participants. No information on reg-

ular medication was available for 14 participants (4.0%); see Table I for further characterization of participants regarding degree of mobility, age and regular medication.

Thromboembolic events in the past were identified in two participants (0.7%). One patient with known cardiac insufficiency and EF of 30% had a history of acute chest pain at the age of 31 years. Pulmonary embolism was confirmed by thoracal computer tomography and elevated D-Dimers. Concomitant deep vein thrombosis of the lower extremities was excluded by sonography and the patient was started on life-long oral anticoagulation. Another patient reported a history of an ischemic insult of the left mid cerebral artery and a left-ventricular thrombus that was diagnosed at the same time. Unfortunately, consent for follow-up of this patient was not available, so that additional information could not be collected. Correlation analysis revealed no significant coherences of past thromboembolism with the degree of mobility, age at event, regular medication, regular intake of steroids or cardiac medication; see Figure 2 for illustration of replies to questions enquiring signs of thrombophilia and bleeding tendency.

Interestingly, four other participants reported a history of past thromboembolic events in the initial survey, but were excluded from respective analysis after medical reports were available:

One patient was found to have a history of a perinatal hemorrhagic stroke with resulting unilateral spastic hemiplegia; according to the available medical information the patient and his mother were both found to have a heterozygous factor V mutation. Two patients (0.6%) reported a history of cerebral fat embolism after precedent femoral fracture at age of 14 and 15 years, respectively. A patent fo-

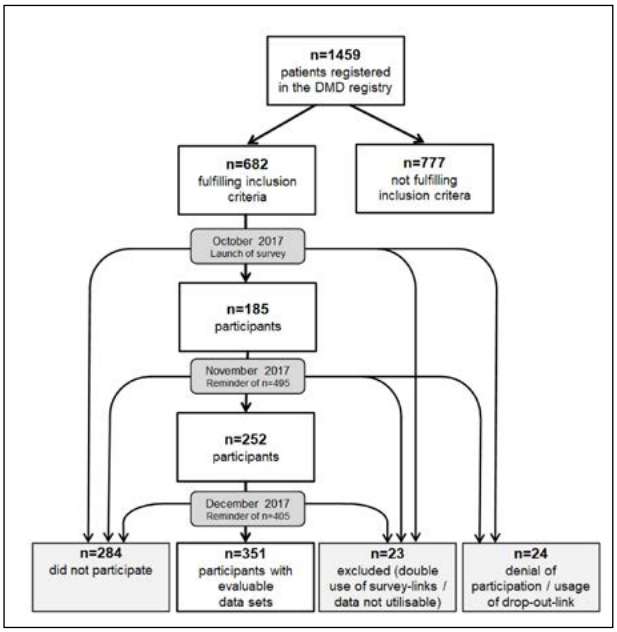

Figure 1. Flowchart of the survey.

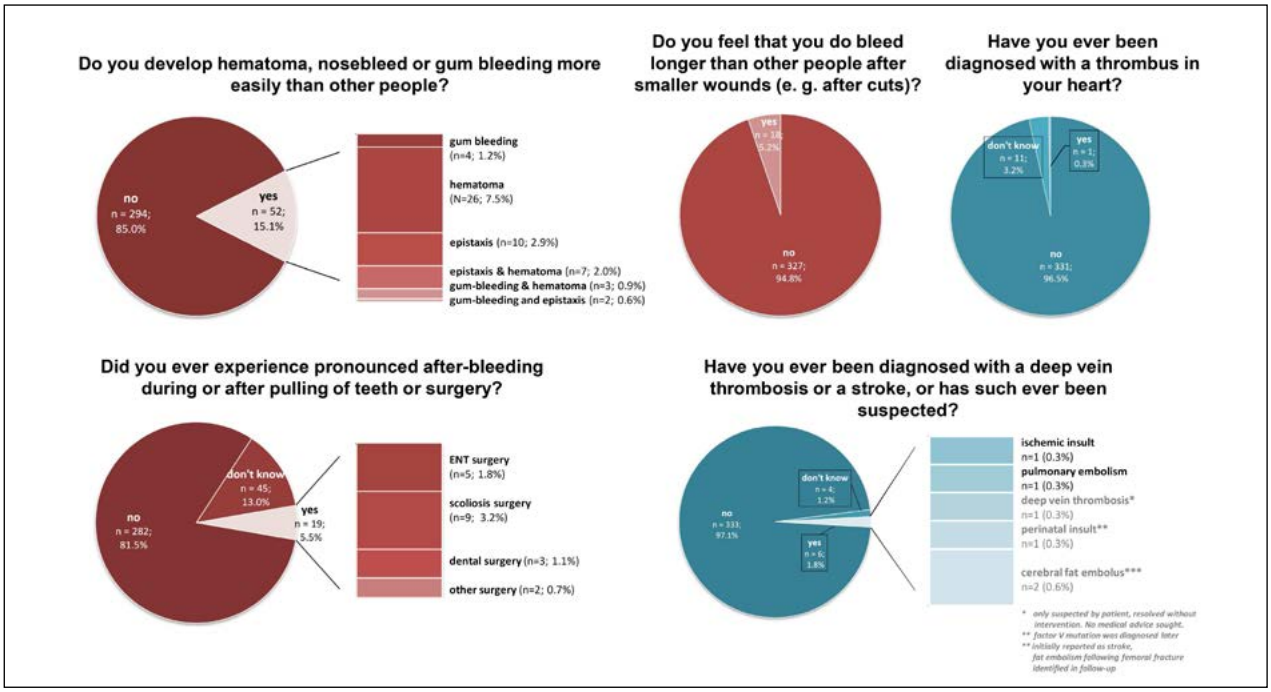

Figure 2. Illustration of questions enquiring signs of thrombophilia and bleeding tendency and given answers by survey participants.

Table I. Characterization of participants by degree of mobility, age and regular medication.

Question	Valid answers	Possible answers	All	Mobility	
				Ambulatory	Non- ambulatory
Degree of mobility					
		Fully ambulatory	n = 110 (31.3%)	-	-
		Wheelchair, partially	n = 37 (10.5%)	-	-
		Wheelchair, predominantly	n = 17 (4.8%)	-	-
		Wheelchair, solely	n = 185 (57.6%)	-	-
	n = 351 (100.0%)	*Bedridden*	n = 2 (0.6%)	-	-
Age					
		< 6 ys	n = 44 (12.5%)	n = 44 (12.5%)	n = 0 (0%)
		6-10 ys	n = 83 (23.6%)	n = 76 (21.6%)	n = 7 (2.0%)
		11-15 ys	n = 72 (20.5%)	n = 31 (8.8%)	n = 41 (11.7%)
		16-20 ys	n = 69 (19.6%)	n = 10 (2.8%)	n = 59 (16.8%)
	n = 337 (96.0%)	*> 20 ys*	n = 69 (19.6%)	n = 0 (0%)	n = 69 (19.6%)
Medication					
		None	n = 78 (22.2%)	n = 34 (9.7%)	n = 44 (12.5%)
		Steroids	n = 147 (41.9%)	n = 111 (31.6%)	n = 36 (10.3%)
		Cardiac medication	n = 102 (29.1%)	n = 15 (4.3%)	n = 87 (24.8%)
		Oral anticoagulation	n = 3 (0.9%)	n = 1 (0.3%)	n = 2 (0.6%)
		Ataluren	n = 17 (4.8%)	n = 12 (3.4%)	n = 5 (1.4%)
	n = 337 (96.0%)	*Eteplirsen*	n = 1 (0.3%)	n = 1 (0.3%)	n = 0 (0%)

ramen ovale could be excluded by echocardiography in both patients. Another single patient reported a suspected deep vein thrombosis in the past, whereas clinical symptoms resolved without therapy and medical advice was not sought.

A bleeding tendency was reported by 76 participants (21.7%). Of those 52 (14.8%) reported a disposition to hematoma, epistaxis or gum bleeding, or a combination of those symptoms. Occurrence of peri- or postoperative hemorrhage or hemorrhage after extraction of teeth was reported by 19 patients (5.4%), and a prolonged bleeding after cuts was declared from 18 patients (5.1%) (see Table III in the supplemental material for an overview of all reported perioperative, postsurgical or post interventional bleeding episodes that were reported). No significant correlations with age, degree of mobility, cardiac medication or preceding or present intake of blood-thinners were found.

Those participants that gave consent for follow-up were contacted for more detailed information. In short, a disposition to bruises was reported more often (79%) than development of nose-bleeding (50%) or gum-bleed (14%).

Bleeding-episodes after smaller wounds were of short duration (< 5 minutes) in most participants (78%) and need for medical intervention to stop bleeding had been necessary in only one patient. See Table IV in the supplemental material for a more detailed synopsis of follow-up data.

Analysis of genetic findings of participants showed a similar distribution of underlying genetic mutations compared to previous studies in larger cohorts of DMD

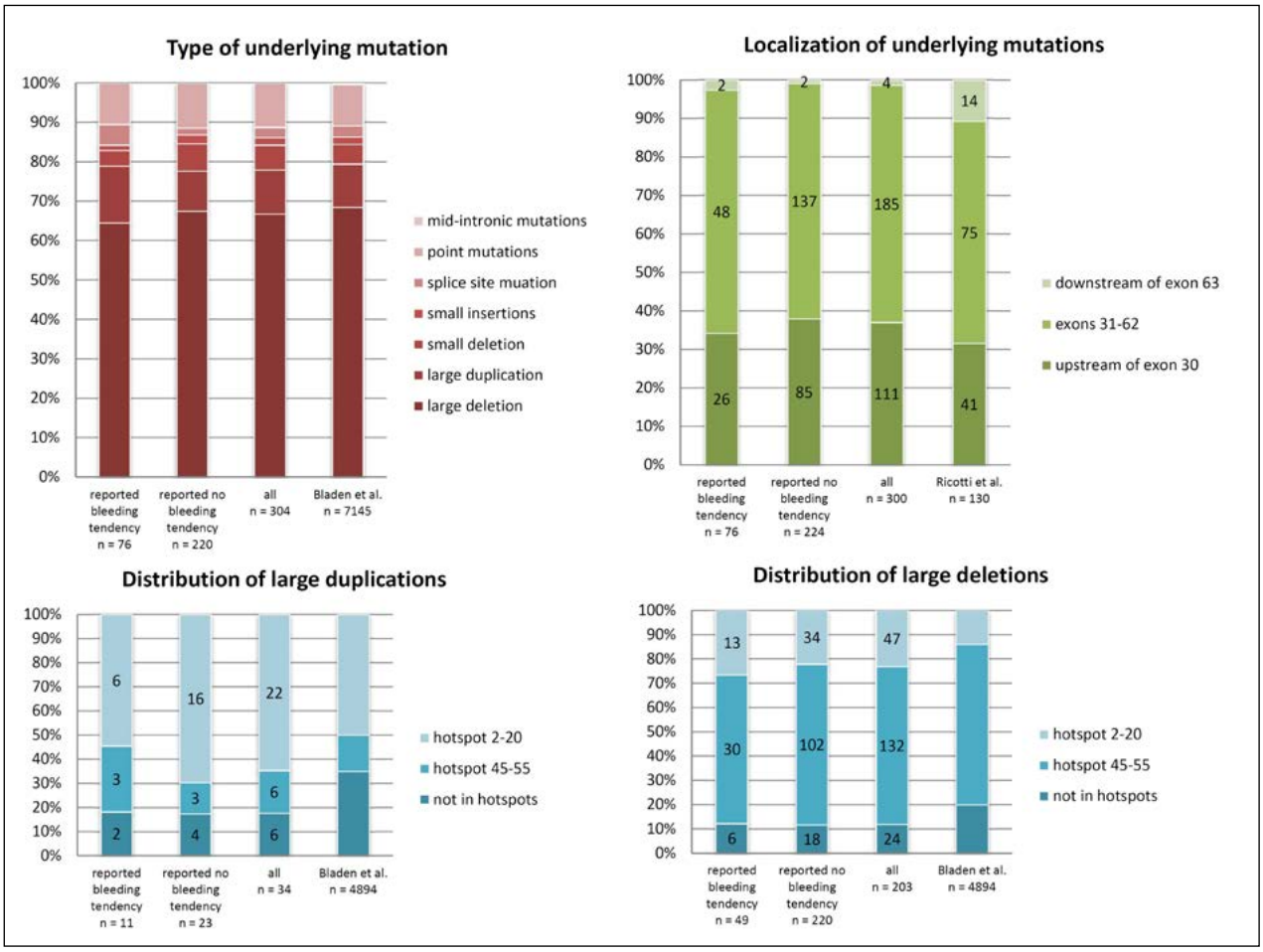

Figure 3. Genetic findings of participants.

patients [15] and did not differ between those patients reporting an increased bleeding tendency and those that did not (see Figure 3). Furthermore, there was no association with mutations affecting the expression of dp71 and a reported bleeding tendency.

Discussion

This study was initiated to assess clinically relevant thromboembolic events or increased bleeding symptoms in patients with DMD. The overall incidence for pediatric venous thromboembolism (VTE) has been estimated between 0.07 to 0.49 per 10,000 children [17-19] and approximately tenfold higher in hospitalized children [20], while the incidence in adulthood has been estimated to be about 5.6-16 per 10,000 adults per year [21]. While the overall incidence of VTE in the pediatric population is lower when neonates are excluded from epidemiologic analyses [22], there is consensus that other typical risk factors include presence of central catheters, intake of oral contraceptive pills, systemic bacterial infection and immobilization [19,23,24]. The role of cardiac disease as a risk factor for VTE is well established in adulthood, but has so far not been highlighted by retrospective analyses in children. Chronic immobility and cardiac insufficiency are well known risk-factors for thromboembolic vascular obstruction – both typical for advanced stages of DMD. Among the initial reports of thromboembolic events in the past were two cases of fat embolism following bone fracture and one case of a perinatal insult with subsequently diagnosed factor V mutation – all three not clearly attributable as primary VTEs without underlying medical condition or risk factor. Another patient was excluded as a real thromboembolism in the past was found to be unlikely as symptoms resolved quickly without intervention. Thus, despite reports of venous thromboembolism in DMD, findings of this registry-based survey do not support an increased risk for thromboembolic events that exceeds the usual risk of immobilized patients due to different medical conditions [19]. An increased risk for

cerebral infarction in DMD has been suggested repeatedly; most recently by authors of a retrospective analysis in which arterial ischemic strokes were identified in 4 out of 54 analyzed patients [9]. However, in this study, only one participant reported a history of left-ventricular thrombus and ischemic insult, so that we do not conclude an increased risk for cerebral infarction in DMD.

In contrast, the high number of patients reporting a bleeding tendency is striking. Bleeding complications can be caused by a primary or a secondary hemostasis defect. Typical symptoms of an impaired hemostasis include prolonged bleeding after injuries, mucocutaneous bleedings, such as epistaxis and gum bleed and hematoma. Secondary hemostasis comprises a complex cascade of different coagulation factors and can be activated either intrinsically or extrinsically. Secondary hemostasis has been investigated in DMD before and these analyses did not show any disease-specific abnormalities [25,26]. The primary hemostasis on the other side relies on platelet function and on the von Willebrand Factor (vWF). A prolonged bleeding time, considered to be a reliable indicator of dysfunctional cellular hemostasis, has been reported in DMD patients in different studies [4,25-27]. Analyses of platelet aggregometry and vWF-antigen, as well as flow cytometry of platelet receptors in patients with DMD gave very heterogeneous and inconclusive results [4,26,27]. As the smaller dystrophin isoform dp71 is also expressed in thrombocytes and has been shown to be important for changes in thrombocytic configuration and contractile properties [13,14], a possible disease-specific impairment of thrombocytic function thus appears possible for DMD. Dp71 is using an alternative promotor upstream of exon 63, but mutations in this part of the dystrophin gene are overall rare in DMD-patients. The analysis of the genetic findings of participants did not reveal differences between those reporting an increased bleeding tendency and those that did not regarding the type or localization of underlying mutations.

Apart from being prone for a possible selection bias, there are other limitations of this cross-sectional survey: The design of the survey did not allow discriminating between primary and secondary hemostasis. For example, the question "Do you develop hematoma, nosebleed or gum bleeding more easily than other people?" embraces both possible disorders of primary (gum bleed, epistaxis) and secondary (bruises) hemostasis. The overall response-rate of the follow-up questionnaires was too low to reliably classify the initial data retrospectively. Furthermore, the design of the questions did not allow for a separate indication of fat embolism, so that two cases of this well-known complication of bone fractures in DMD were initially reported as 'stroke' and correctly identified only in follow-up. Finally, reporting an increased bleeding tendency is certainly not the same as really suffering from it. The approach by questionnaire was chosen to capture the self-evaluation of DMD patients and to assess bleeding events in daily life that do not necessarily lead to medical attendance and thus may not be detectable by review of medical records. As in every self-reporting questionnaire based survey, this approach carries a risk of imprecise data.

Further research is needed to clarify whether a disease-specific dysfunction of coagulation is associated with the phenotypic spectrum in DMD. The results of this survey do not prompt a DMD-specific risk for thromboembolic events exceeding the risk of typical thrombophilia-associated conditions such as immobility or cardiac insufficiency. DMD patients are known to have higher blood losses during scoliosis surgery. Results of this survey suggest an additional bleeding tendency in daily life of DMD-patients that is not determined by type or localization of the underlying genetic mutations.

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Appendix

Table II. Questionnaire with indication of all questions in full-text and results according to degree of mobility.

	Question		Valid answers	Possible answers	All	Mobility	
					Ambulatory	Non- ambulatory	
	#1	**"How do you move in daily life?"**	n = 351 (100.0%)	*Ambulatory*	n = 110 (31.3%)	-	-
				Wheelchair, partially	n = 37 (10.5%)	-	-
				Wheelchair, predominantly	n = 17 (4.8%)	-	-
				Wheelchair, solely	n = 185 (57.6%)	-	-
				Bedridden	n = 2 (0.6%)	-	-
	#2	**"Do you develop hematoma, nosebleed or gum bleeding more easily than other people?"**	n = 346 (98.6%)	*No*	**n = 294** (83.8%)	n = 132 (37.6%)	n = 162 (46.2%)
				Yes	**n = 52** (14.8%)	n = 29 (8.3%)	n = 23 (6.5%)
	#3	**"Did you ever experience pronounced after-bleeding during or after pulling of teeth or surgery (e. g. spinal surgery, tonsillectomy, adenotomy)?"**	n = 346 (98.6%)	*I don't know*	**n = 45** (12.8%)	n = 25 (7.1%)	n = 20 (5.7%)
				No	**n = 282** (80.3%)	n = 133 (37.9%)	n = 149 (42.4%)
				Yes	**n = 19** (5.4%)	n = 3 (0.8%)	n = 16 (4.6%)
	#4	**"Do you feel that you do bleed longer than other people after smaller wounds (e. g. after cuts)?"**	n = 345 (98.3%)	*No*	**n = 327** (93.2%)	n = 154 (43.9%)	n = 173 (49.3%)
				Yes	**n = 18** (5.1%)	n = 7 (2,0%)	n = 11 (3.1%)
Bleeding tendency	**"Yes" in questions 2, 3 or 4?**				**n = 76 (21.6%)**	n = 34 (9.7%)	n = 42 (11.9%)

	#5	**"Have you ever been diagnosed with a thrombus in your heart in former examinations (heart ultrasound)?"**	n = 343 (97.7%)	*I don't know*	**n = 11** (3.1%)	n = 7 (2.0%)	n = 4 (1.1%)
				No	**n = 331** (94.3%)	n = 154 (43.9%)	n = 177 (50.4%)
				Yes	**n = 1** (0.3%)	n = 0 (0%)	n = 1 (0.3%)
	#6	**"Have you ever been diagnosed with a deep vein thrombosis or a stroke, or has such ever been suspected?"**	n = 343 (97.7%)	*I don't know*	**n = 4** (1.1%)	n = 2 (0.5%)	n = 2 (0.5%)
				No	**n = 333** (94.9%)	n = 159 (45.3%)	n = 174 (49.6%)
				Yes	**n = 6** (1.7%)	n = 0 (0%)	n = 6 (1.7%)
Thrombophilia	**"Yes" in questions 5 or 6?**				**n = 6 (1.7%)**	n = 0 (0%)	n = 6 (1.7%)
	#7	**"Have you taken blood-thinning medication in the past?"**	N = 343 (97.7%)	*I don't know*	**n = 337** (96.0%)	n = 161 (45.9%)	n = 176 (50.1%)
				No	**n = 6** (1.7%)	n = 0 (0%)	n = 6 (1.7%)
	#8	**"Which medication do you take frequently?"**	N = 337 (96.0%)	*None*	**n = 78** (22.2%)	n = 34 (9.7%)	n = 44 (12.5%)
				Following (selected free-text indications):	**n = 259** (73.8%)	n = 127 (36.2%)	n = 132 (37.6%)
				Steroids	**n = 147** (41.9%)	n = 111 (31.6%)	n = 36 (10.3%)
				Cardiac medication	**n = 102** (29.1%)	n = 15 (4.3%)	n = 87 (24.8%)
				Oral anticoagulation	**n = 3** (0.9%)	n = 1 (0.3%)	n = 2 (0.6%)
				Ataluren	**n = 17** (4.8%)	n = 12 (3.4%)	n = 5 (1.4%)
				Eteplirsen	**n = 1** (0.3%)	n = 1 (0.3%)	n = 0 (0%)
	#9	**"How old are you?"**	N = 337 (96.0%)	*< 6 ys*	**n = 44** (12.5%)	n = 44 (12.5%)	n = 0 (0%)
				6-10 ys	**n = 83** (23.6%)	n = 76 (21.6%)	n = 7 (2.0%)
				11-15 ys	**n = 72** (20.5%)	n = 31 (8.8%)	n = 41 (11.7%)
				16-20 ys	**n = 69** (19.6%)	n = 10 (2.8%)	n = 59 (16.8%)
				> 20 ys	**n = 69** (19.6%)	n = 0 (0%)	n = 69 (19.6%)

Table III. Synopsis of reported events of perioperative and postsurgical hemorrhage in the initial survey.

Patient ID	Type of surgery	Medical letter accessible?	Age at surgery	Comments
Orthopedic surgery (n = 9)				
423	Spondylodesis Th4-L5	Yes	15	Postsurgical transfusion of red blood cells
456	Spondylodesis Th3-L4	Yes	14	Anamnestic report of postsurgical hemorrhage. No respective findings in medical letter, apart from wound healing deficits
631	Spondylodesis Th5-L5	Yes	14	Postsurgical transfusion of red blood cells
659	Spondylodesis Th3 -Th5, sublaminar fusion Th6-Th12, pedicle screws L1-S1	Yes	14	Intraoperative and postsurgical transfusion of red blood cells
661	Spondylodesis Th3-S1	Yes	20	Postoperative hematothorax, transfusions of red blood cells and plasma, substitution of FXIII and antithrombin. Diagnosis of FXIII-deficiency was made postoperative.
437	Spondylodesis Th3-S1	Yes	15	Intraoperative transfusion of red blood cells
323	Spondylodesis	No	NA	NA
404	Spondylodesis	No	NA	NA
599	Spondylodesis	No	NA	NA
ENT surgery (n = 4)				
192	Tonsillectomy adenotomy, paracentesis	Yes	4	Increased bleeding with need for surgical control on postoperative day #2, transfusion of red blood cells
377	Tonsillectomy	Yes	5/6	Increased bleeding with need for surgical control on postoperative day #11
290	Tonsillectomy	No	NA	Increased bleeding with need for surgical control on postoperative day #3
326	Tonsillectomy	No	NA	NA
Dental surgery (n = 3)				
223	Molar tooth extraction	No	4	No need for medical intervention
417	Dental surgery (2 episodes)	No	3 and 14	No need for medical intervention
433	Molar tooth extraction	No	17	Anamnestic report of wound healing deficiency and need for antibiotic therapy
Other types of surgery (n = 2)				
549	Frenuloplasty, phimosis surgery	Yes	18	Anamnestic report of post-surgical bleeding, no respective findings in medical letter

NA = information not available

Table IV. Synopsis of follow-up data for patients giving account of an increased bleeding tendency in the initial survey.

	Question	Positive answer in initial survey	Consent for follow-up	Follow-up (response-rate)	Method of follow-up	Questions in follow-up:	(Possible) answers	
Bleeding tendency	**Frequent hematoma, nosebleed or gum bleeding?**	n = 52	n = 42	n = 24 (57.1%)	*Questionnaire*	"Do you develop bruises more easily than others?"	Yes	N = 19 (79%)
							No	N = 5 (21%)
						"Do you develop nose-bleed more easily than others?"	Yes	N = 12 (50%)
							No	N = 12 (50%)
						"Do you develop gum-bleed more easily than others?"	Yes	N = 3 (14%)
							No	N = 18 (86%)
	Pronounced after-bleeding after pulling of teeth or surgery?	n = 17	n = 14	n = 14 (100.0%)	*Phone call/ medical letters*	Dental surgery	Molar extraction	N = 5 (35%)
							Dental procedure	N = 4 (26%)
						Spinal surgery	Spondylodesis	N = 6 (43%)
						ENT surgery	Tonsillectomy/ adenotomy	N = 5 (36%)
						Others	Phimosis surgery	N = 1 (7%)
	Prolonged bleeding?	n = 18	n = 14	n = 9 (64.3%)	*Questionnaire*	"How many episodes of prolonged bleeding after cuts do you have per year?"	< 1/year	N = 4 (44.4%)
							1-5/year	N = 4 (44.4%)
							6-12/year	N = 0 (0%)
							> 12/year	N = 0 (0%)
						"How long lasts an episode in average?"	< 5 minutes	N = 7 (77.8%)
							> 5 minutes	N = 2 (22.2%)
						"Has ever been need for medical measures to stop bleeding?"	Yes	N = 8 (88.9%)
							no	N = 1 (11.1%)

Note that more cases of bleeding complications after dental surgery were reported in follow-up than in the initial survey.

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Identification, molecular characterization and segregation analysis of a variant *DMPK* pre-mutation allele in a three-generation Italian family

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DM1 is an autosomal dominant multisystemic disease caused by an unstable CTG repeat expansion in the 3'-untranslated region (UTR) of the *DMPK* gene. The complex variant *DMPK* expanded the alleles containing CAG, CCG, CTC and/or GGC interruptions repetition sequences have been reported in 3-8% of DM1 patients. To date, very few information is available about the frequency and clinical consequences of pre-mutated *DMPK* variant allele. In this study, we describe a three-generation Italian family showing the segregation of an interrupted *DMPK* allele within the premutation range. TP-PCR with primers complementary to CCG repetitions and direct sequencing allow us to identify a hetero-triplet $(CTG)_6(CCGCTG)_{15}(CTG)_5$ repeat structure. The haplotype analysis demonstrated that this variant allele is associated with the European founder DM1 haplotype. The pyrosequencing analysis of the CpG islands contained in the flanking regions of the CTG array, did not show the presence of a *cis effect* of the CCG interruptions on the methylation profile of the DM1 locus. The analysis of both meiotic transmissions, one maternal and one paternal, revealed the intrafamilial stability of the DM1 premutation among relatives. Our findings further support the hypothesis of a stabilizing effect of CCG interruptions on the mutational dynamics of the DM1 locus, also in intermediate *DMPK* alleles.

Key words: *DMPK* variant alleles, premutation, TP-PCR analysis, methylation

Introduction

Myotonic dystrophy type 1 (DM1, OMIM #160900) is the most common form of adult muscular dystrophy, with a prevalence of 12.5/100,000 and an autosomal dominant mode of inheritance [1,2].

Patients with DM1 show a progressive multisystemic disease affecting mainly skeletal muscle, heart and the central nervous system [3]. DM1 is caused by the expansion of an unstable CTG trinucleotide repeat located in the 3′ untranslated region of the *DMPK* gene, on chromosome 19q13.3 [4,5]. The number of CTGs is polymorphic in the general population, with a range of 5 to about 37 repeats, a premutation range from 38 to 49 triplets

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Conflict of interest
The Authors declare no conflict of interest

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and increases to 50 and up to many thousand in patients [6]. Germline instability is the major factor determining the pronounced anticipation seen in DM1 and depend on the sex of the transmitting parent. Intermediate alleles can be stably inherited for several generations, especially if transmitted by the mother, passages through male germline almost invariably lead to a large increase into the full disease range [7,8]. On the contrary, alleles longer than 80 CTGs tend to expand when transmitted through affected mothers and, depending on the mutation size, may lead to the congenital form of the disease. In the last years, variant (CAG)n, (CCG)n, (CTC)n and (CGG)n repeats interspersed within the CTG array have been reported, with an overall frequency of about 3-8% in DM1 patients [9-13]. These variant alleles greatly alter the mutational dynamics and the phenotypic manifestation of the disease, leading to important practical consequences on DM1 genetic testing and counseling. Interruptions of the repeated tract have been observed in normal and intermediate alleles of other trinucleotide repeats (TNRs) diseases, such as spinocerebellar ataxia 1 (SCA1) and fragile X syndrome, where one or more interruptions must be lost before expansion can occur. In DM1, the search for variant repeats in a large set of *DMPK* normal alleles did not reveal any interruptions, which instead have been detected only within the intermediate alleles of four individuals with discordant clinical phenotypes [14-17]. The analysis of a larger set of individuals is therefore warranted to assess the frequency and the possible causal or modifying effect on DM1 phenotype of variant *DMPK* intermediate alleles.

In this work we describe a three-generation Italian family in which a single 41 repeats interrupted allele showing a $(CTG)_6(CCGCTG)_{15}(CTG)_5$ configuration segregates. Interestingly, the length and interruption pattern of this allele remained stable through either paternal and maternal transmissions, with no apparent consequences on the phenotype. The haplotype and methylation analysis of the DM1 locus demonstrated its association with the European founder DM1 haplotype and the absence of *in cis* epigenetic effects on the genomic region surrounding the CTG array.

Materials and methods

Patients collection and DNA extraction

Samples were obtained from an Italian family referred to the Genetic Unit of the Children's University Hospital "A. Meyer", Florence for DM1 and PWS genetic testing. Major clinical data available from patients and their pedigree are summarized in Figure 1. Genomic DNA was extracted from peripheral blood leukocytes us-

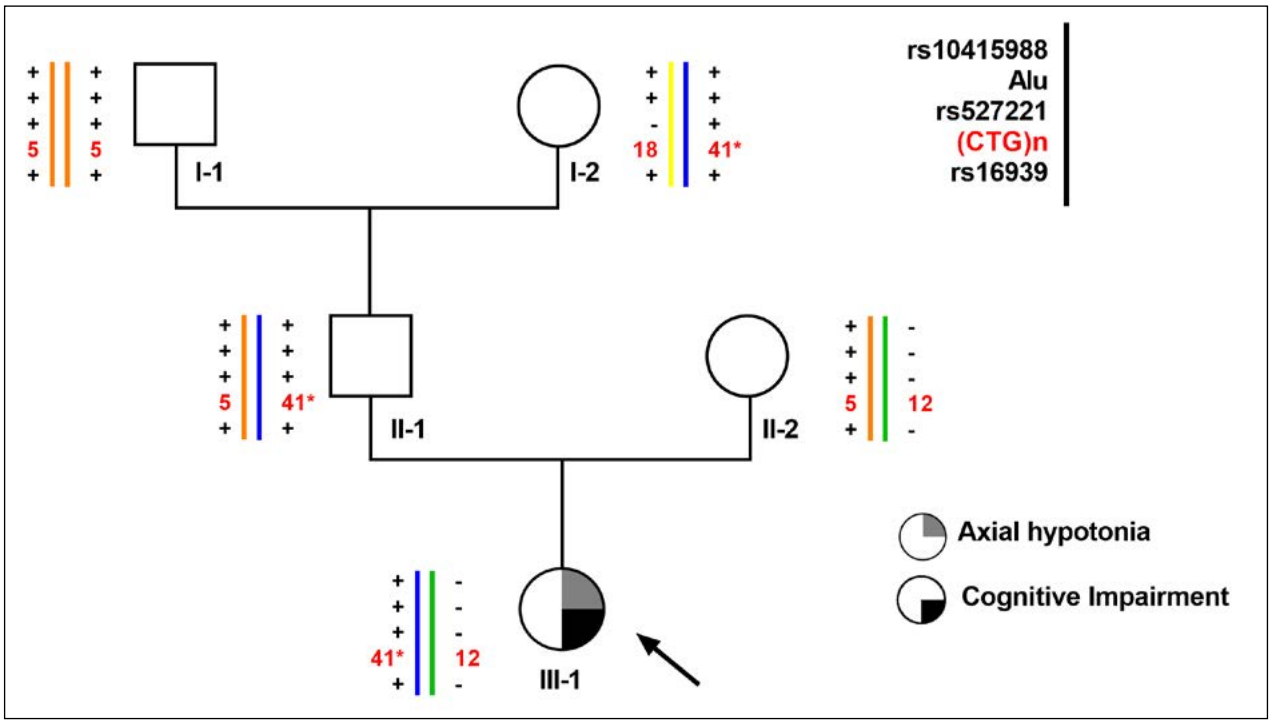

Figure 1. Pedigree and haplotype analysis of the DM1 locus of our three-generation Italian family. The proband is marked with an arrow.* This value corresponds to the apparent number of CTG repetitions.

ing EZ1 DNA Blood Kit (Qiagen, Germany) according to standard procedures. Informed consents were obtained from all individuals participating to this study (Ethical Approval register number: CS_02/2019).

Short range PCR (SR-PCR), triplet repeated-primed PCR (TP-PCR) and direct sequencing of DMPK alleles

DNA of each patient was analyzed and characterized using SR-PCR and TP-PCR with synthetic fluorescently labeled primers flanking and within the CTG repetitions, as described [16]. The interruptions of the CTG array were detected with P4 internal primers, specific for the variant motif (CCGCTG)n, according to published protocols [14]. The characterization of the interruption motifs was obtained using Sanger direct sequencing of gel-purified (Gel Extraction kit, Qiagen, Germany) SR-PCR products corresponding to the premutated *DMPK* alleles. Direct sequencing was performed using the BigDye Terminator Cycle Sequencing Ready Reaction kit and the sequences were analyzed with ABI 3130xl Automated Sequencer (ThermoFisher Scientific, Massachusetts, USA).

Haplotype analysis

The haplotype analysis of the DM1 locus was performed using four biallelic polymorphic markers as previously described [18]. The presence of a 1-kb *Alu* insertion/deletion polymorphism was typed by PCR using a three-primers protocol [19]. The following additional 3 single base pair polymorphic markers rs10415988 [Taq1] (T/C) in 15kbCEN, rs527221 [Bpm1] (G/C) in *DMPK* exon 10 and rs16939 [HinfI] (T/G) in intron 9 were typed by PCR and Sanger direct sequencing.

DMPK methylation analysis

Bisulfite conversion DNA (1 µg in 20 µl) has been obtained using EZ DNA Methylation-Gold Kit (Zymo Research, Irvine, CA, USA) according to the manufacturer's instructions. The genomic DNA was quantified by the Qubit® 2.0 Fluorometer (ThermoFisher Scientific, Massachusetts, USA).

The methylation study was carried out on *DMPK* genomic region as previously described [20]. A 189 bp fragment (10 CpG sites) in 5' end region of CTG array was amplified by PCR from bisulfite-treated DNA using CTCF-1F (5'- GGAAGATTGAGTGTTCGGGGTA -3') and CTCF-1R (5'- Biotinylated -GGGTTTTTGTAGTCGGGAATG -3') primers. For 3' end region, a 173 bp fragment (6 CpG sites) was amplified using CTCF-2F (5'- TAAATTGTAGGTTTGGGAAG -3') and CTCF-2R (5'-biotyinylated- GGGAAATTTGTTTTTGTTAAA -3') primers. PCR conditions: 95°C for 5 min, followed by 50 cycles of 95°C for 30 sec, 55°C for 30 sec and 72°C for 30 sec with final extension of 5 min at 72°C. The pyrosequencing analysis was performed on a PyroMark Q24 (Qiagen, Germany) with following sequencing primers: CT1-S (5'- GGGTTTTCGTTTAGTTTTAGTTTTG -3') for 5' end and CTCF-2F for 3' end regions. The methylation percentage at each CpG sites was quantified by the PyroMark Q24 software, version 2.0.7 (Qiagen, Germany).

Results

Phenotypes

The proband (Subject III-1) is the 16 year-old-second-born female of a couple of healthy and unrelated parents. The perinatal period was characterized by a marked axial hypotonia, minor hypertonia in the lower limbs, respiratory insufficiency, poor sucking, and frequent apneas. She had facial hypotonus and micro-retro-gnathia. A child-neuropsychiatrist evaluation diagnosed a medium/severe cognitive impairment, with relational and learning problems at 6 years of age. No myotonic phenomenon was elicited in the proband nor in her parents on physical examination. Her father (II-1) is a 54 years old ex-sportsman (still practicing amateur marathon) and has a normal electromyography (EMG). The paternal grandmother (I-2) is 84 years old and reported only a senile cataract with no other signs of DM1. According to the proband's clinical phenotype, the genetic testing for Prader-Willi syndrome (PWS) – and DM1 as differential diagnosis – was requested for III-1. The PWS/AS-region analysis showed a maternal uniparental heterodisomy (UPD) of chromosome 15 (data not shown), confirming the diagnosis of PWS in the proband.

Detection of DMPK premutated alleles and characterization of variant non CTG interruptions

As SR-PCR analysis of the proband's DNA showed a 12 and an apparent 41 CTG alleles at the DM1 locus, the *DMPK* molecular analysis was extended to all the available family members. The results (see Figure 1), indicated that only the proband's father and the grandmother (II-1 and I-2 respectively) were carriers of the 41 triplets *DMPK* allele. In order to exclude the presence of a DM1 expansion not detectable with SR-PCR analysis, a bi-directional TP-PCR, with primers P4-CTG (3'- end of CTG array) and P4-CAG (5'- end of the CTG array) was then performed. TP-PCR analysis did not reveal the presence of a pathological CTG expansion of the *DMPK* gene in subjects I-2, II-1 and III-1. However, the electrophoresis profiles were atypical, characterized by gaps in the continuous 3-base-pairs ladder signal, strongly suggesting an atypical interruption

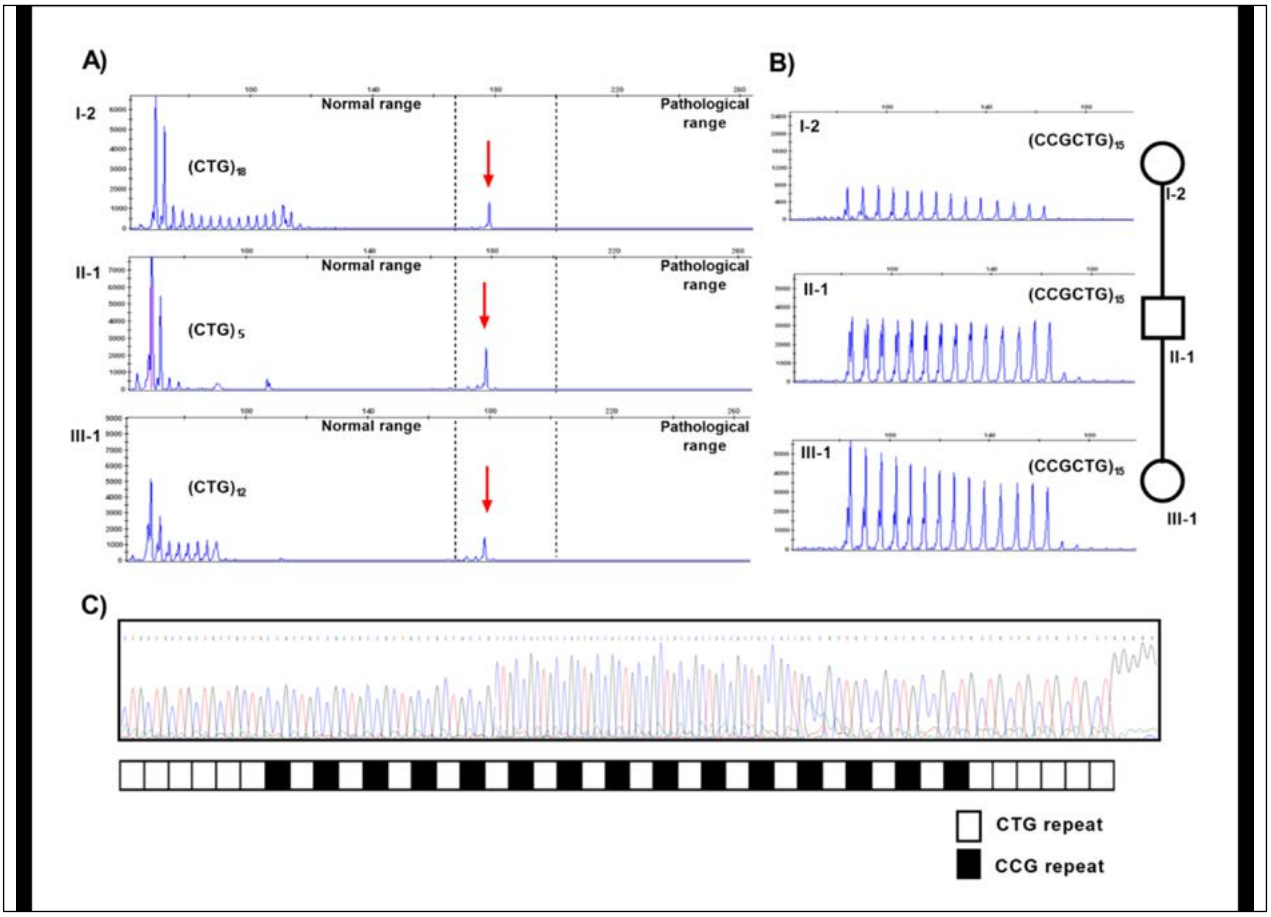

Figure 2. Molecular characterization of the DM1 locus: TP-PCR profiles of *DMPK* alleles of I-2, II-1 and III-1 with primers complementary to the CTG (A) and CCG (B) repetitions. Red arrows indicate the amplification of the apparent (CTG)41 *DMPK* allele. C) Sequencing analysis and structure of *DMPK* premutated interrupted alleles. Each square represents a single CCG repeat.

within the CTG repeated array (Figure 2A). A second round of TP-PCR, using P4-CCGCTG primer, confirmed the presence of atypical CCG interruption which remains stable through the meiotic transmissions (Figure 2B). The Sanger sequencing of the SR-PCR products confirmed a $(CTG)_6(CCGCTG)_{15}(CTG)_5$ (HGVS nomenclature: NM_001081563.2: c.*224_*283CTG[6]CCGCTG[15] CTG[5]) interruption motif in the proband (III-1), in her father (II-1) and in the grandmother (I-2) (Figure 2C).

Haplotypes analysis

To determine the haplotype in linkage disequilibrium with the DM1 interrupted premutated alleles, all family members were analyzed with 4 genetic markers closely flanking the (CTG)n repeat: rs16936 in intron9 [HinfI], rs527221 in exon10 [BpmI], rs10415988 [TaqI] and 1-Kb Alu insertion/deletion. Their polymorphisms were used to construct compound haplotypes as follow: TaqI site present (+)/absent (-); 1-kb Alu insertion (+)/deletion (-); BpmI site present (+)/absent (-) and HinfI site present (+)/absent (-).As a result, three possible haplotype combinations were identified in this family: haplotype (+ + + +) linked to the $(CTG)_5$ and $(CTG)_6(CCGCTG)_{15}(CTG)_5$ *DMPK* alleles, haplotype (- - - -) linked to $(CTG)_{12}$ allele, and haplotype (+ + - +) linked to the $(CTG)_{18}$ allele (Fig. 1).

CpG methylation profile

In order to test a possible *in cis* effects of the CCG interruptions on the *DMPK* locus already reported in DM1 patients [20], we performed a methylation analysis on two CpG islands flanking the unstable CTG tract. The first CpG island, that contains 10 CpG sites, was localized in the upstream region of the (CTG)n expansion, while the second CpG island was in the downstream region of CTG array including 6 CpG sites (Fig. 3).

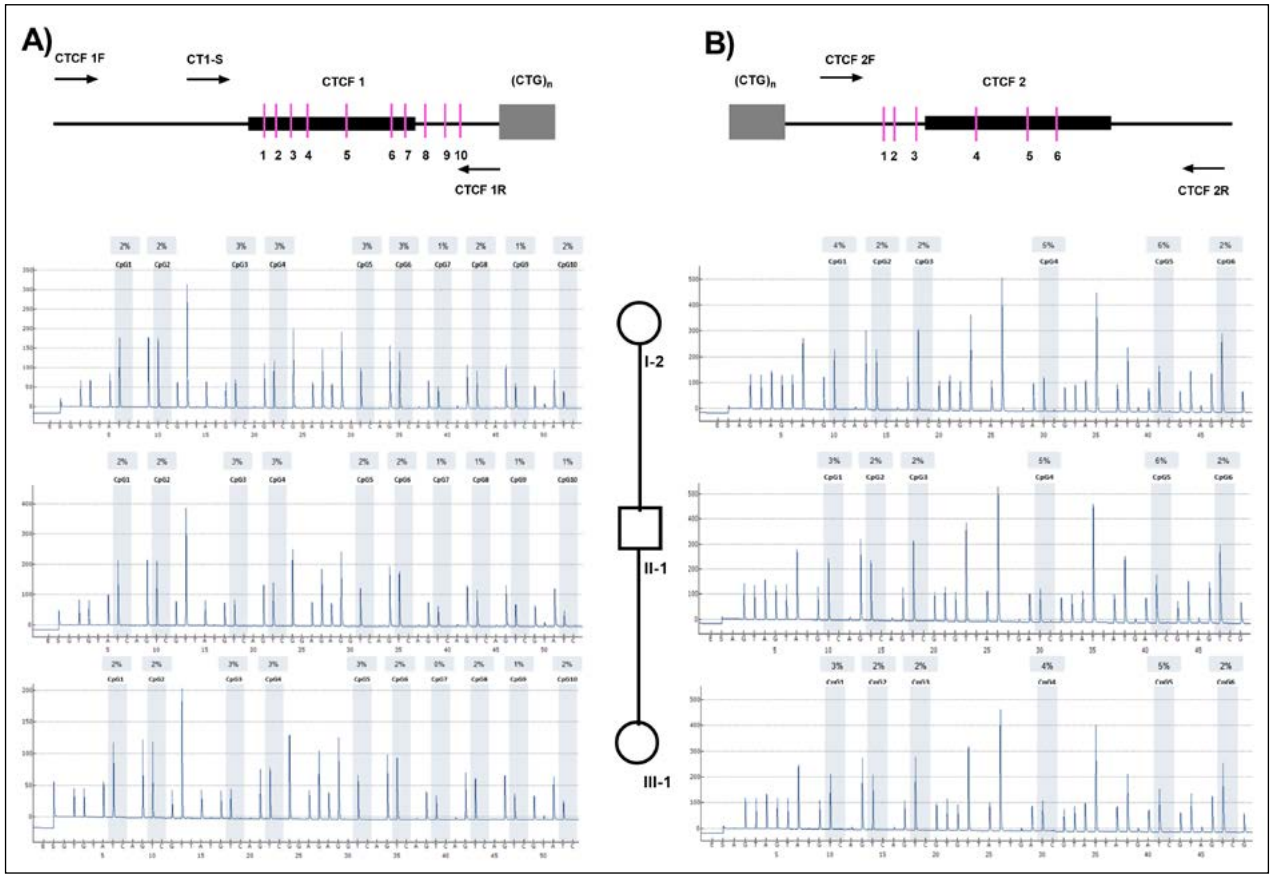

Figure 3. Methylation analysis of regions 5' and 3' to the CTG array. Up-panel: genomic structure upstream (A) and downstream (B) of the CTG repeat in the *DMPK* gene. The CpG islands are represented as bars, CTCF1 binding site as black box, CTG repeat region as gray box and the PCR primers used in this study are indicated as arrows. Down-panel: Pyrosequencing profiles of I-2, II-1 and III-1 samples, respectively 5' (A) and 3' (B) regions to the CTG array.

By pyrosequencing analysis, a homogeneous 2-4% hypomethylation level with no significant differences among I-2, II-1 and III-1 samples was found in the upstream region (Fig. 3A). Equally, in the downstream region of the (CTG)n expansion an average methylation level of 3% with no significant differences in I-2, II-1 and III-1 individuals was found (Fig. 3B). These results indicate that the presence of CCG interruptions in the DM1 locus do not influence the methylation levels of the genomic regions flanking the (CTG)n array.

Discussion

The presence of atypical interruptions in the DM1 locus can influence the phenotype in several simple repeat expansion disorders [11]. To date, very few patients have been described not carrying CTG interruption in DM1 alleles containing more than 35 repetitions and the effects on the mutational dynamics and phenotypic outcome are still subject of debate. The first *DMPK* interrupted allele was described by Leeflang et al. [17], in a sperm donor carrying apparent 37 CTG repetitions and a $(CTG)_4(CCGCTG)_{16}(CTG)$ hetero-triplet structure. Musova et al. [14], reported three individuals with a similar repeated structure in intermediated *DMPK* alleles. However the contribution to the phenotype remains unclear because of the simultaneous occurrence of other neuromuscular conditions [14]. Our proband has been also referred for DM1 genetic testing because of a neuromuscular phenotype, which was associated to a maternal uniparental heterodisomy of PWS/AS critical region on chromosome 15q, confirming the diagnosis of PWS. The molecular characterization of the DM1 locus revealed the presence of a premutated *DMPK* allele with apparent 41 CTG repetitions in a heterozygous state. The combined use of SR-PCR and bidirectional TP-PCR allows us to detect CCG interruptions of the CTG tract with a $(CTG)_6(CCGCTG)_{15}(CTG)_5$ structure. The extension of the molecular analysis in all the available family members established that the premutated interrupted *DMPK* allele was paternally inherited and derived from the proband's grand-

mother. The interruption we have defined may explain the anomalous meiotic stability of this allele through both maternal and paternal transmission, as demonstrated by direct sequencing of SR-PCR products in II-1 and I-2 DNA samples. The linkage analysis also showed that the $(CTG)_6(CCGCTG)_{15}(CTG)_5$ *DMPK* allele (HGVS nomenclature: NM_001081563.2: c.*224_*283CTG[6]CCGCTG[15]CTG[5]) is associated with the same chromosomal haplotype as pathogenic alleles present in affected DM1 patients (defined A-haplotype) [21]. We can speculate that the hexamer at the DM1 locus originated from at least one mutation of the CTG to CCG, combined with subsequent slippage of the hexamer. The novelty of our work report is that no clinical signs of DM1 have been detected in the proband's father and grandmother, in an apparent normal clinical status despite their advanced age. This allow us to conclude that the *DMPK* $(CTG)_6(CCGCTG)_{15}(CTG)_5$ premutated allele does not have phenotypic consequences in the analyzed individuals. Our family enlarges the set of individuals so far described who carry the variant *DMPK* premutations and may help to assess the frequency and the possible clinical effects of these very rare alleles.

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Late-onset MADD: a rare cause of cirrhosis and acute liver failure?

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Late-onset multiple acyl-CoA dehydrogenase deficiency (MADD) is a severe inborn error of fat metabolism. In late-onset MADD, hepatopathy in the form of steatosis is commonplace and considered a benign and stable condition that does not progress to more advanced stages of liver disease, however, progression to cirrhosis and acute liver failure (ALF) has been reported in two previous case reports. Here, we report a 22-year-old man, who suffered from late-onset MADD and died from cirrhosis and ALF. In the span of three months repeated clinical examinations, blood tests, and diagnostic imaging as well as liver biopsy revealed rapid progression of hepatopathy from steatosis to decompensated cirrhosis with portal hypertension. Routine studies for recognized etiologies found no evident cause besides MADD. This case report supports the findings of the two previous case reports and adds further evidence to the suggestion that late-onset MADD should be considered a rare cause of cirrhosis and ALF.

Key words: multiple acyl-CoA dehydrogenase deficiency, MADD, cirrhosis, acute liver failure

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Introduction

Multiple acyl-CoA dehydrogenase deficiency (MADD; OMIM 231680) is an autosomal recessive inherited disorder of the mitochondrial electron transfer flavoprotein (ETF) chain causing dysfunction of fatty acid, amino acid, and choline metabolism [1]. The clinical presentation varies widely from fatal, neonatal-onset phenotypes to milder, late-onset phenotypes [2]. Patients with late-onset MADD commonly present with liver steatosis and lipid storage myopathy as well as recurrent episodes of hypoglycemia and metabolic decompensation due to their impaired fat oxidation and consequently greater dependence upon carbohydrate oxidation [3]. Treatment of MADD is to prevent attacks of metabolic decompensation by means of dietary fat and protein restriction along with supplementation of riboflavin and L-carnitine, while the mainstay of hypoglycemic attacks is oral or intravenous glucose [4]. The liver steatosis in these patients is considered a benign and stable condition that does not progress to more advanced stages of liver disease and thus does not need specific treatment. However, since 2013, two patients with late-onset MADD have been described with inexplicable, sudden, and rapid progression of hepatopathy to cirrhosis and acute liver failure (ALF) [5,6].

Here, we present a third late-onset MADD patient, who suddenly and rapidly progressed to decompensated cirrhosis with portal hypertension and died from acute liver failure (ALF) with no evident cause besides MADD.

Case report

A 22-year-old man suffered from late-onset MADD. He was born from healthy parents, who were of Palestinian ethnicity and first cousins. He was the fourth of seven siblings. His three older siblings had all died from MADD at ages 10 months, 3 years, and 10 years respectively, in emergencies of acute hypoglycemia and metabolic decompensation. Of his three younger siblings, the oldest, aged 21, also suffers from MADD, but is well treated by fat and protein restriction and supplementation with riboflavin and L-carnitine. On this treatment, she has not experienced any recurrent episodes of hypoglycemia or metabolic decompensation, but she does have liver steatosis and lipid storage myopathy. For further details, she is described as patient #2 in the study by Madsen K. L. et al. [7]. The last two siblings do not suffer from MADD.

The parents received genetic counseling and were given prenatal diagnostic testing. DNA sequencing of placental tissue from chorionic villus sampling was performed and the index patient and his affected siblings were diagnosed with MADD subsequent to the finding of a novel homozygous c.1074G > C variant in the *ETFDH* gene leading to a substitution of the evolutionary conserved arginine in position 358 of the ETF dehydrogenase with serine. The variant leads to an expressed ETF dehydrogenase and is associated with a reduction of myristate oxidation at about 40% [8].

From birth, the index patient was closely followed with biannual routine exams at the Centre for Inherited Metabolic Diseases. He was given the standard treatment of diet along with supplements of riboflavin and L-carnitine. Especially as a smaller child, he had numerous crises of hypoglycemia and metabolic decompensation often preceded by various trivial infections. All incidents were treated successfully with intravenous glucose. On a few occasions he advanced in metabolic decompensation to an extent that lead to acute kidney failure and acute respiratory distress syndrome needing hemodialysis and assisted ventilation, but he always made a full recovery. The routine exams showed no other manifestations of MADD than moderate liver steatosis and lipid storage myopathy causing mild exercise intolerance as well as muscle weakness and pain. As expected for MADD, he had consistently – though rather varied – elevations of plasma-creatine kinase (250-2500 U/L; normal 22-198 U/L) and an acyl-carnitine profile with normal concentration of free carnitine and markedly increased concentrations of acyl-carnitines of all lengths. He never showed any symptoms or signs of any other disease.

At age 21, after several years without having experienced a single hypoglycemic event, he was hospitalized with acute hypoglycemia and metabolic acidosis. Investigations revealed intestinal Clostridium difficile infection (CDI) as the root cause and he soon recovered on intravenous glucose and antibiotics. However, in the following four months he was hospitalized and treated for acute hypoglycemia and metabolic acidosis two more times due to recurrent CDI. At the third admission, he also presented with abdominal pain and therefore a CT scan of the abdomen was performed (Figs. 1A-B). The scan was normal except for a significantly enlarged spleen measuring 18 cm in craniocaudal length (normal < 13 cm) with otherwise normal morphology. Following the third hospitalization he underwent fecal microbiota transplantation, which ended his recurrent CDI.

Nonetheless, two months later he was admitted to the hospital in a state of hypoglycemia and metabolic decompensation for the fourth time. This time investigations revealed septicemia caused by two staphylococcal bacteria (s. epidermidis and s. capitis). Once again he was treated with glucose and antibiotics. He recovered well but one month later he was hospitalized due to two weeks of progressive abdominal pain, bloating, and mushy stools. On clinical exam, he was jaundiced, febrile, and his abdomen was notably distended. Blood tests showed prolonged coagulation and elevated transaminases and bilirubin suggesting impaired synthesis function and acute necrosis of liver cells. A new CT scan of the abdomen was performed (Figs. 1C-D). It showed a cirrhotic-looking liver containing multiple small hypodense lesions implying parenchymal micro-abscesses along with clear signs of liver decompensation in the form of large-volume ascites and rectal portosystemic shunt with collaterals to the inferior mesenteric vein. Consistent splenomegaly and severe colitis were also found. On clinical and biochemical reevaluation, he had no stigmata of chronic liver disease and routine hepatitis tests were negative. Profiles of amino acids and acylcarnitines remained unchanged. Ultrasound images and CT angiography of the liver further revealed patent vessels and no biliary dilatation but the most severe degree of portal hypertension with hepato-fugal portal venous flow. The liver biopsy showed parenchymal damage with severe macro- and micro-vesicular steatosis along with accumulation of glycogen, copper, and Mallory bodies superimposed on a cirrhotic liver. There were no signs of malignancy or infection.

Hereafter the patient's liver function rapidly deteriorated and he died two months later in the intensive care unit from ALF. In this period the patient was not a candidate for neither liver transplant surgery or trans-jugular intrahepatic portosystemic shunt insertion as his short-term and long-term survival chances were considered too poor. His family declined autopsy and thus the patient could not be further examined.

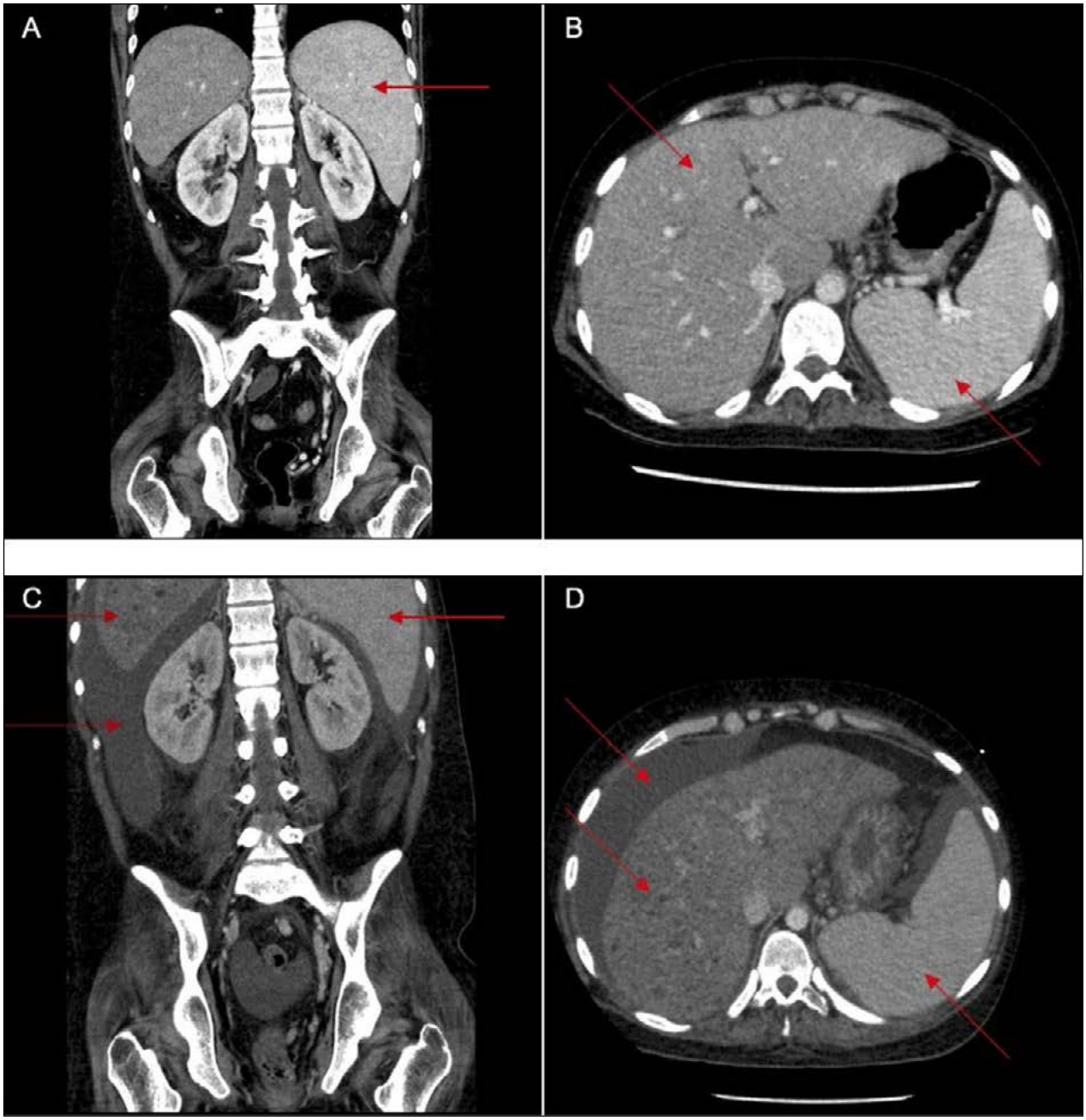

Figure 1. CT scan of the abdomen. A) Coronal scan showing normal intraabdominal conditions except for an enlarged spleen measuring 18 cm in craniocaudal length (arrow); B) Transverse scan showing normal liver and spleen tissues (arrows); C) Coronal scan showing multiple small hypodense lesions in the liver, ascites, and consistent splenomegaly (arrows); D) Transverse scan showing normal spleen tissue, but multiple small lesions in the liver tissue and ascites (arrows).

Discussion

We report the third case of a patient suffering from late-onset MADD, who suddenly and rapidly progressed in habitual hepatopathy from the state of steatosis to cirrhosis with portal hypertension, with no other evident cause than MADD. Our patient showed no other symptoms or signs of any other disease throughout his life except for a moderately enlarged spleen and episodes of recurrent CDI and septicemia, all this was observed and occurred prior to the diagnosis of cirrhosis with portal hypertension. CDI and septicemia are not associated with the development of cirrhosis, but splenomegaly is

frequently seen alongside cirrhosis [9]. Thus, it is most obvious that the enlarged spleen was simply a result of severe steatosis or beginning cirrhosis of the liver, and the infections merely caused by an overall immunocompromised state as seen in advanced liver disease, rather than to manifestations of another coexisting disease. Moreover, the two previously reported patients did not have either splenomegaly or events of infections prior to the onset of cirrhosis, indicating that these findings were most likely without significance to the development of cirrhosis in our patient. Our patient's liver biopsy showed accumulation of copper that could suggest Wilson's disease, but accumulation of copper is also seen in cholestatic liver disease as a consequence of decreased biliary excretion of copper [10]. Our patient had cholestasis with hyperbilirubinemia well explaining copper accumulation. Furthermore, he had no sign of Kayser-Fleischer ring. Therefore, further diagnostic testing for Wilson's disease was deemed unnecessary. All in all, the presence of another undiagnosed disease as a root cause or aggravating factor of the progressive liver disease in our patient cannot be ruled out with absolute certainty, but it must be considered highly unlikely.

The two previously reported patients – a German and a Chinese – who had the same age (22 years), were otherwise healthy and showed basically the same onset and timeframe of disease progression to cirrhosis and ALF as our patient. However, only the Chinese man eventually died, while the German man had a partial recovery with riboflavin and ubiquinone therapy [5,6]. Unlike our patient who had been diagnosed prenatally and closely followed with comprehensive routine exams throughout his life, the others were unaware to suffer from MADD until their first symptoms and signs of liver disease appeared, and were subsequently diagnosed with MADD. In this way, our case report establishes with greater certainty that the onset was sudden and progression rapid in this very rare presentation of MADD. It is striking and interesting that all three cases are 22 years old, at onset. Aside from a fortuitous coincidence, we believe this simply indicates that the patients were able to compensate for gradually increasing liver steatosis during their youth and early adulthood. When the degree of steatosis eventually became too high for the liver, cirrhosis developed rapidly. Our case report, together with the two previous case reports, shows that late-onset MADD can be a rare cause of sudden and rapid development of cirrhosis and ALF. This should be kept in mind when following and treating patients with MADD. Therefore, basic biannual clinical and biochemical tests are recommended, and in case of signs of declining liver function, further investigations should be promptly conducted.

Initially our patient presented with recurring hypoglycemic events, many years after his last event in childhood. They were attributed solely to his simultaneous infections, but it is possible that they were – in part or in whole – actually caused by the decline in liver function and the impairment of glycogenolysis and gluconeogenesis, hence MADD patients depend heavily.

Patients with late-onset MADD have habitually elevated transaminases due to their lipid storage myopathy, but an increase in transaminases is also seen in beginning liver failure due to acute necrosis of liver cells. When our patient presented with recurrent hypoglycemic events, the increase in transaminases was entirely ascribed to the lipid storage myopathy, and therefore liver biopsy was not performed. However, it is likely that some part of the increase might have represented an early stage in development of cirrhosis. Thus, in these cases it would be an expedient measure to investigate the isoenzymes of the transaminases, in order to distinguish between if they are predominantly derived from muscle or liver tissue. If derived from the liver tissue, the liver biopsy is indicated to diagnose at an early stage the progression of the disease and to initiate the appropriate treatment as soon as possible.

In conclusion, this case supports the findings of the two previous case reports and adds further evidence to the suggestion that late-onset MADD should be considered as a rare cause of cirrhosis and ALF.

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Laing early-onset distal myopathy with subsarcolemmal hyaline bodies caused by a novel variant in the *MYH7* gene

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Myopathies caused by *MYH7* gene mutations are clinically and pathologically heterogeneous and, until recently, difficult to diagnose. The availability of NGS panels for hereditary neuromuscular diseases changed our insight regarding their frequency and allowed a better perception of the different phenotypes and morphological abnormalities associated.
We present a male Portuguese patient with the classical phenotype of Laing early-onset distal myopathy (MPD1) beginning at 6 years of age, very slowly progressive, and with a mild to moderate impact on daily life by the age of 56. Muscle biopsy showed a myopathic pattern with hyaline bodies and cores. The NGS panel for structural myopathies identified a novel missense heterozygous variant, c.T4652C (p.Leu1551Pro), in the exon 34 of the *MYH7* gene.

Key words: *MYH7* gene variant, laing early-onset distal myopathy, subsarcolemmal hyaline bodies

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Introduction

The *MYH7* gene, located at chromosome 14, encodes the slow/b-cardiac myosin heavy chain (MyHC I), a class II myosin expressed in cardiomyocytes and type 1 striated muscle fibers [1]. Cardiac and skeletal muscle diseases can be caused by *MYH7* gene mutations, the former being much more common [1]. Most of the *MYH7* gene mutations responsible for cardiomyopathies are located in the globular head domain of the protein, while mutations causing myopathies tend to be localized in the distal region of the rod domain [2-4]. Almost all reported *MYH7* gene mutations are dominant [1,2], with very few cases reported of autosomal recessive inheritance [5].

MYH7-related myopathies are less rare than once expected [6]. Based on clinical and morphological data, they are classified as myosin storage myopathy (MSM) and Laing early-onset distal myopathy (MPD1), the latter much more common [6,7]. While MPD1 is characterized by very distal lower limb muscle weakness [8] and variable morphological data, MSM is defined by specific subsarcolemmal accumulation of hyaline bodies (HB), with a predominant limb-girdle clinical phenotype [9].

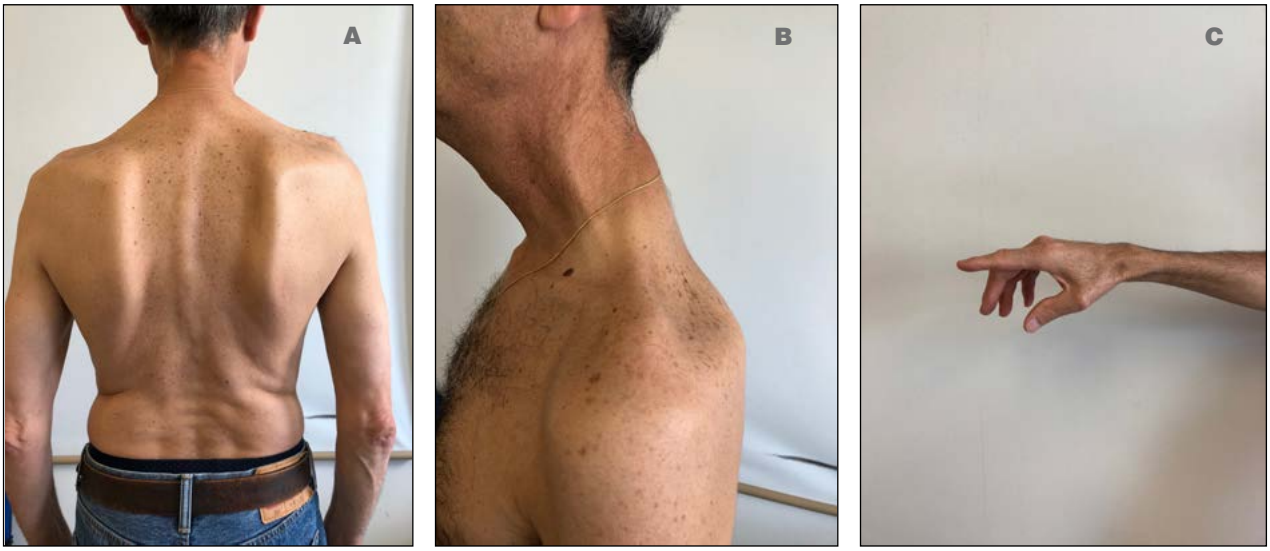

Figure 1. Muscle atrophy of the shoulder girdle muscles (trapezius, supraspinatus and infraspinatus) (A, B). Finger-drop with preserved extension of the second finger (C).

Herein, we present a male Portuguese patient with a *MYH7*-related myopathy caused by a novel missense heterozygous variant, c. 4652T > C (p. Leu1551Pro), in the exon 34 of *MYH7* gene.

Clinical case

The patient is a 56-year-old man, the single offspring of a non-consanguineous Azorean couple. His father and brother were suspected of having a similar muscular condition, but were not available to examination.

At the age of 6 years, he reported walking difficulties, probably with minor impact on daily life in the following years, since he was admitted to the military service by the age of 18. At 30 years of age, he reported difficulty in raising both arms and, at the age of 40 years, he developed weakness of the extensor muscles of the fingers.

Neurological examination at 52 years of age revealed muscle atrophy of the shoulder girdle muscles (Figs. 1A-B), particularly of the trapezius, supraspinatus and infraspinatus, and of the posterior muscles of the forearm and of the tibial anterior muscles, bilaterally. There was no calf hypertrophy. At rest, the scapula was displaced posteriorly and, with arm abduction, it moved laterally and inferiorly. There was bilateral foot-drop with "big-toe dropping", Achilles tendon retraction and bilateral finger-drop with preserved extension of the second finger (Fig. 1C). The patient walked with a steppage gait and walking on tip-toes was possible. Gowers maneuver was negative. Muscle strength evaluation of the upper limbs revealed severe symmetrical weakness of the long extensor muscles of the thumb and of the 3rd to 5th fingers (0/5 MRC), and weakness of the long extensor muscle of the 2nd finger, abductor pollicis brevis, abductors muscles of the arm and of the infraspinatus muscle (4-/5 MRC). In the lower limbs, there was bilateral severe weakness of the tibial anterior muscle and of the long extensor muscle of the hallux (0/5 MRC) and weakness of the extensor muscles of the 2nd to 4th toes (3/5 MRC). Cervical flexion was weak (4-/5 MRC). Myotatic reflexes were abolished throughout. Sensory examination and cranial nerves evaluation were normal.

Complementary exams

Laboratory workout was repeatedly performed and revealed normal CK values. Cardiac evaluation via echocardiogram and evaluation of respiratory function were unremarkable. Electrophysiologic studies showed normal peroneal (recording from the EDB muscle) and sural nerve conduction velocities and amplitudes. Muscle needle examination showed the presence of repetitive complex discharges on the tibial anterior muscles with sparse fibrillations potentials and positive waves. No motor unit potentials could be activated. Motor unit potentials of short amplitude and duration, and polyphasic, were observed on the right vastus medialis and extensor indicis muscles.

Muscle biopsy

Deltoid muscle biopsy revealed a myopathic pattern with variation in fiber size with marked atrophy and hypertrophy, increased internalized nuclei and mild interstitial fibrosis (Fig. 2A). Some muscle fibers had hyaline

material, mildly eosinophilic with the hematoxylin and eosin stain (Fig. 2B) and pale-green with the Gomori thricrome, without reactivity for oxidative enzymes, suggestive of hyaline bodies. Multiple fibers presented irregularity of the intermyofibrillar reticulum and occasional core formation (Fig. 2C). Pronounced type 1 predominance with very rare type 2 fibers was observed (Fig. 2D). Subsarcolemmal hyaline material lacked immunoreactivity for desmin (Fig. 2E).

Molecular study

The next generation sequencing (NGS) panel for structural myopathies was performed through a custom targeted NGS panel. Enrichment was performed by in-solution hybridization and, after library preparation, the DNA library was subjected to NGS.

Variants that passed the quality control step were prioritized according to their minor allele frequencies (MAF < 0.01) in the following databases: 1000G, Exome Aggregation Consortium (ExAC), Exome Variant Server (EVS), Genome Aggregation Database (gnomAD), and our in-house population database (IberDB). Possible pathogenicity of the missense variants detected was assessed using the in silico tools CONDEL[3] researchers have developed various methods and their related computational tools to classify these missense SNVs as probably deleterious or probably neutral polymorphisms. The outputs produced by each of these computational tools are of different natures and thus difficult to compare and integrate. Taking advantage of the possible complementarity between different tools might allow more accurate classifications. Here we propose an effective approach to integrating the output of some of these tools into a unified classification; this approach is based on a weighted average of the normalized scores of the individual methods (WAS, GERP++[4] (4)such as protein-coding exons, noncoding RNAs, and regulatory sequences that control the transcription of genes. However, these functional sequences are embedded in a background of DNA that

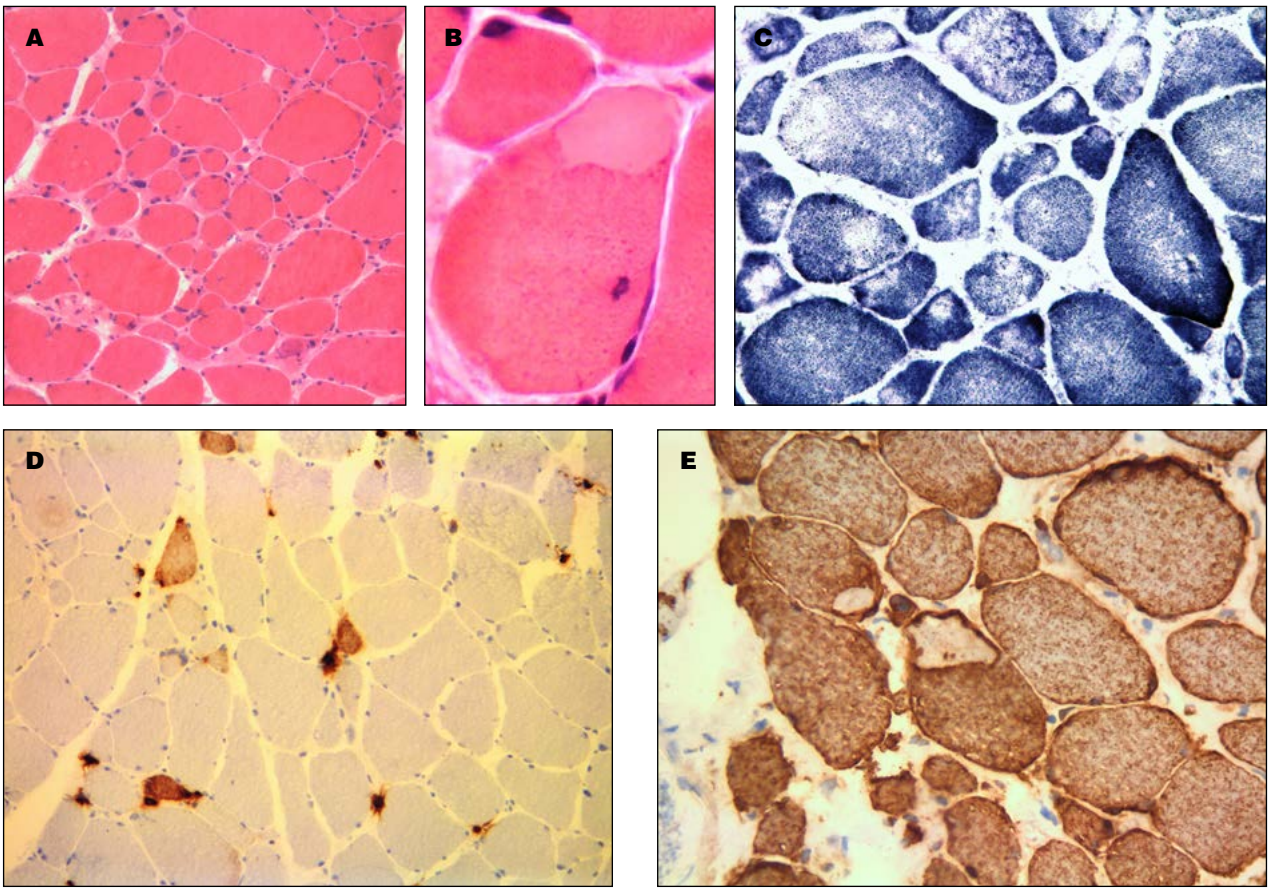

Figure 2. Deltoid muscle biopsy. Muscle fiber size variability with marked atrophy and hypertrophy and fibers (H&E – 100x) (A); with subsarcolemmal hyaline material (*) (H&E – 400x) (B); rare core lesions (*) (SDH – 200x) (C); pronounced type 1 fiber predominance with only few scattered type 2 fibers (fast myosin immune – 100x) (D); subsarcolemmal material without immunoreactivity for desmin (*) (400x) (E).

serves no discernible function. Thus, a major challenge in the field of genomics is the accurate identification of functional sequences in the human genome. One approach to identify functional sequences is to align the genome sequences of many divergent species and search for sequences whose similarity has been maintained during evolution. We have developed GERP++, a software tool that utilizes this "comparative genomics" approach to identify putatively functional sequences. Given a multiple sequence alignment, GERP++ identifies sites under evolutionary constraint, i.e., sites that show fewer substitutions than would be expected to occur during neutral evolution. GERP++ then aggregates these sites into longer, potentially functional sequences called constrained elements. Using GERP++ results in improved resolution of functional sequence elements in the human genome and reveals that a higher proportion of the human genome is under evolutionary constraint (~7%, and CADD [5].

A missense heterozygous variant, c.4652T > C (p. Leu1551Pro), was detected in the exon 34 of *MYH7* gene (Fig. 3).

This *MYH7* gene sequence variant was not registered in the 1000G, Exome Aggregation Consortium (ExAC), Exome Variant Server (EVS), Genome Aggregation Database (gnomAD), our in-house population database (IberDB) or in ClinVar associated with disease. This residue is highly conserved (GERP score 4,55) and bioinformatic analysis with CONDEL (score: 0,75) and CADD (score 29,9) suggests that this variant is potentially deleterious.

Discussion

Classical phenotypes associated with *MYH7* gene mutations include the limb-girdle and distal myopathic phenotypes, firstly describe in 1971[9] and 1995[8], respectively. In the following years, different clinical presentations were identified and in 2016, there was a proposal to group them into 3 forms of *MYH7*-related myopathies: 1- early onset form of distal myopathy with cores; 2- late onset form of distal myopathy without cores and variable association with cardiomyopathy and/or fiber type disproportion and 3- limb-girdle involvement with myofibrillar damage resembling MSM [6].

The current perspective on *MYH7*-related myopathies is that there is a continuum in the clinical presentation of the different clinical phenotypes, in some way related to the age of onset of the disease [6].

The pathological findings followed a similar evolution in classification, firstly with a dichotomous division comprising the presence of subsarcolemmal hyaline bodies, characteristic of MSM, or the presence of non-specific abnormalities with predominance of type I fibers, typical of

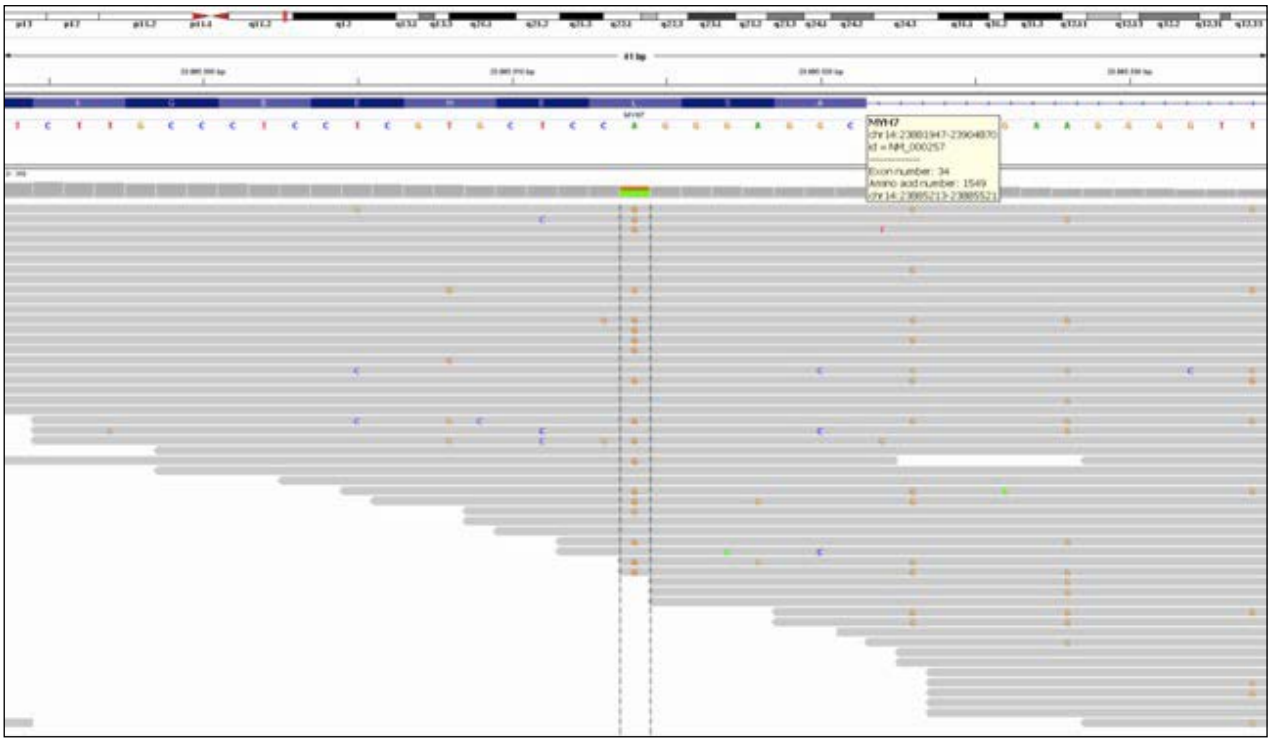

Figure 3. I.G.V. (integrative genomics viewer): the missense heterozygous variant, c.4652T > C (p. Leu1551Pro), in the exon 34 of *MYH7* gene, detected in the case report.

MPD1 [10]. Later on, other pathological patterns were consistently identified: congenital fiber-type disproportion and central core disease, most commonly seen in MPD1 [6,7,11].

In the first descriptions of *MYH7*-related cardiomyopathies and myopathies, there were no reports on the simultaneous occurrence of both diseases in the same patient. At that time, this was explained by the different locations of the *MYH7* gene mutations causing cardiac or skeletal diseases: mutations in the NH globular head were associated with cardiomyopathy, whereas mutations in the COOH tail domain were more prone to cause myopathy. However, more recently, it became clear that mutations in either location could cause either disease, and both could coexist in the same patient [12].

This clinical case has the classical MPD1 phenotype, namely: 1) clinical onset in the distal muscles of the lower limbs, followed by involvement of the shoulder girdle muscles and later, weakness of the long extensor muscles of the fingers; 2) very slowly disease progression; 3) muscle weakness compatible with an ambulant and active life, fifty years after the first symptoms, at a time when cardiac involvement is still absent. Furthermore, the location of the *MYH7* gene variant in exon 34 is characteristically associated to MPD1.

The pathological findings, namely the presence of hyaline bodies, which are atypical or absent in MPD1 cases, together with the novel *MYH7* gene variant here reported, grant uncommon and interesting features to this clinical case.

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CASE REPORTS

Facioscapulohumeral muscular dystrophy (FSHD) and multiple sclerosis: a case report

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Facioscapulohumeral muscular dystrophy 1 (FSHD1) is an autosomal dominant neuromuscular disorder, associated with reduction of tandemly arrayed repetitive DNA elements D4Z4 (DRA), at 4q35. Few cases, especially carriers of 1-3 DRA show a syndromic form. Anecdotally the association of FSHD with multiple sclerosis (MS) is reported. Herein we report a 33 years old Caucasian with a molecular diagnosis of FSHD1 with classical phenotype (clinical category A2) and concomitant white matter lesions suggestive of MS. White matter lesions in patients with FSHD have often been described but rarely investigated in order to evaluate a possible diagnosis of MS. We think that MS and FSHD remain clearly distinct diseases, but growing evidences show a widespread and variable activation of the immune system in patients suffering from FSHD probably an hypotheses on a potential common pathogenetic mechanism between these two disorders could should be better investigated.

Key words: autoimmunity, myopathy, central nervous system

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Introduction

Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominant neuromuscular disorder characterized by progressive weakness of muscle in the face, shoulder girdle and arms. FSHD1 has been associated with reduction of tandemly arrayed repetitive DNA elements, D4Z4, at 4q35. Alleles with 1-3 D4Z4 repeats are generally associated with a syndromic form with extramuscular manifestations (i.e. central nervous system involvement with mental retardation and hearing loss) [1]. To our knowledge, there are only two cases, whose one is an autopsy case [2], of FSHD associated with multiple sclerosis [3]. Herein, we report another case of concomitant multiple sclerosis (MS) in a patient with FSHD1.

Case report

We evaluated a 33 years old Caucasian man referring a short episode of blurred vision in right eye occurs five months before and spontaneously resolved in fortnight period. At our visit he informs us that two years ago, over the course of 2-3 weeks, he developed also lower limb hypoesthesia and diplopia. However, he had a previous genetically confirmed diagnosis of FSHD1 (molecular analysis of DNA documented intermediate deletion

on 4q35 with 3 D4Z4 repeats). The muscle impairment started in the second decade of life with inability to bury the eyelashes, difficulty in pursing the lips, as well as whistling or puffing out the cheeks, winging of the scapulae and bilateral foot drop. In fact his neurologic examination showed a classic FSHD phenotype (Fig. 1), clinical category A2 [4]. Anyway, he presented right Babinski sign, moderate impairment of deep sensation and mild spasticity in the lower limbs, too. As there was centre nervous system involvement patient underwent a MRI brain that showed multiple demyelinating lesions both in the supratentorial and infratentorial white matter and several demyelinating lesions of the spinal cord. None of them demonstrated gadolinium enhancement (Fig. 2). White matter brain MRI alterations suggested a diagnosis of multiple sclerosis (MS). Then visual evoked potentials revealed mild delayed latencies bilaterally. Somatosensory and motor evoked responses both for upper and lower limb were altered [5-7].

Spinal fluid was acellular with normal protein and glucose level; however, oligoclonal bands were detected in CSF electrophoresis only, but not in the serum. Subsequently, the patient underwent an extensive work-up

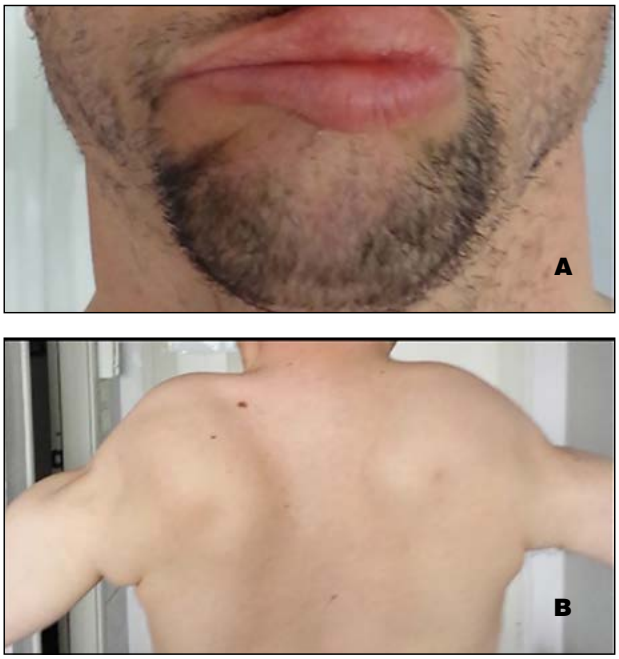

Figure 1. Weakness of lips' (A) and shoulder muscles (B) configuring a classic clinical phenotype of Facioscapulohumeral muscular dystrophy (clinical category A2).

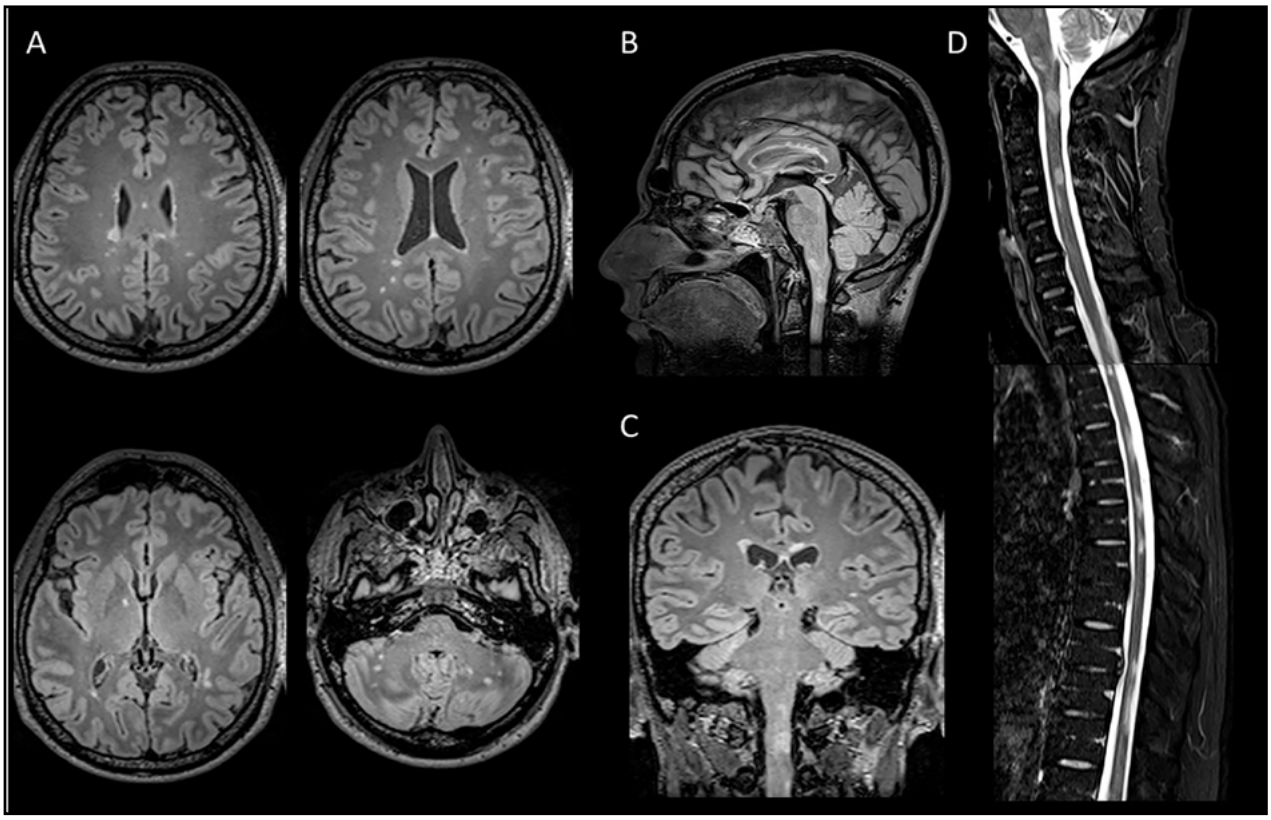

Figure 2. Axial FLAIR images (A) show multiple demyelinating lesions both in the supratentorial and infratentorial white matter. Sagittal (B) and coronal (C) FLAIR sequence depicts corpus callosum and medulla involvement. Sagittal STIR (D) demonstrates several demyelinating lesions of the spinal cord.

searching for metabolic, immune-mediated, infectious and vascular disorders that might simulate the working diagnosis of MS and all tests were either negative or normal. Neuropsychological evaluation using standard battery [8,9] showed no abnormalities. On the other hand, nerve conduction studies were within normal limits [10], whereas needle electromyography confirmed a myopathic pattern consistent with his previous diagnosis of FSHD. Finally, the patient fulfilled the Mc Donald criteria for the diagnosis of MS [11]. He started a disease modifying treatment with dimetilfumarate and non-pharmacological treatments were applied in an attempt to recover disability [12,13].

Discussion

White matter lesions in patients with FSHD have often been described but rarely investigated or correlated with the clinical and/or laboratory phenotype in order to evaluate a possible diagnosis of multiple sclerosis [14].

An autoptic case and a clinical case of association between FSHD and MS have been described [2,3] and hypotheses on a potential common pathogenetic mechanism between these two different disorders has been proposed. Obviously, MS and FSHD remain clearly distinct diseases, but growing evidences show a widespread and variable activation of the immune system in patients suffering from FSHD. Pathological data in muscle biopsy in FSHD patients are consistent with a significant presence of CD8+ T cells both in perivascular and endomysial infiltrates, together with macrophages. Moreover, an increased percentage of circulating CD14+T-bet+ cells and an increased spontaneous production of IL12/IL23p40, IFNγ, TNFα, IL6 and IL10 by PBMC have been described [15]. Furthermore, the presence of demyelinating lesions that have the characteristics of dissemination over time and space suggesting the diagnosis of multiple sclerosis, are increasingly noted in patients suffering from other genetically defined muscular diseases.

However, given the high prevalence of multiple sclerosis, we consider that the association between these two entities in our patient is casual. In the presence of signs or symptoms suggestive of involvement of the CNS in a primitively muscular pathology or the occasional finding of lesions of the white matter in the brain MRI, should always orientate a deep diagnostic screening in order to exclude a possible comorbidity with diseases such as multiple sclerosis.

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Myotonic dystrophy type 1 and high ventricular vulnerability at the electrophysiological evaluation: ICD yes or not?

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A significant number of sudden death (SD) is observed in myotonic dystrophy (DM1) despite pacemaker implantation and some consider the ICD to be the preferential device in patients with conduction disease. According to the latest guidelines, prophylactic ICD implantation in patients with neuromuscular disorder should follow the same recommendations of non-ischemic dilated cardiomyopathy, being reasonable when pacing is needed. We here report a case of DM1 patient who underwent ICD implantation even in the absence of conduction disturbances on ECG and ventricular dysfunction/fibrosis at cardiac magnetic resonance. The occurrence of syncope, non-sustained ventricular tachycardias at 24-Holter ECG monitoring and a family history of SD resulted associated with ventricular fibrillation inducibility at electrophysiological study, favouring ICD implantation. On our advice, DM1 patient with this association of SD risk factors should be targeted for ICD implantation.

Key words: implantable cardioverter defibrillator, myotonic dystrophy type 1, sudden death

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Introduction

Myotonic dystrophy type 1 (DM1) is the most frequent muscular dystrophy in adults. Cardiac involvement is reported in about 80% of cases, even in asymptomatic patients [1]. Conduction system disturbances on surface ECG or ventricular dysfunction are considered the most relevant risk factors for sudden cardiac death (SD), favouring pacemaker (PM) implantation according to latest guidelines [2,3]. However, recent data showed that SD, striking up to one third of patients, can also occur for ventricular tachyarrhythmias (VTA) and that other predictive factors like syncope, family history of SD or non-sustained VT should be taken into account for risk stratification [4]. We report the case of a mildly symptomatic DM1 patient who underwent ICD implantation for high ventricular vulnerability at the electrophysiological evaluation even in the absence of either conduction disturbances at ECG or ventricular dysfunction/fibrosis at non-invasive evaluation by echocardiogram and cardiac magnetic resonance.

Case report

A 42 year-old woman, affected by poorly symptomatic DM1 presented at our emergency department for syncope and palpitations. She was diag-

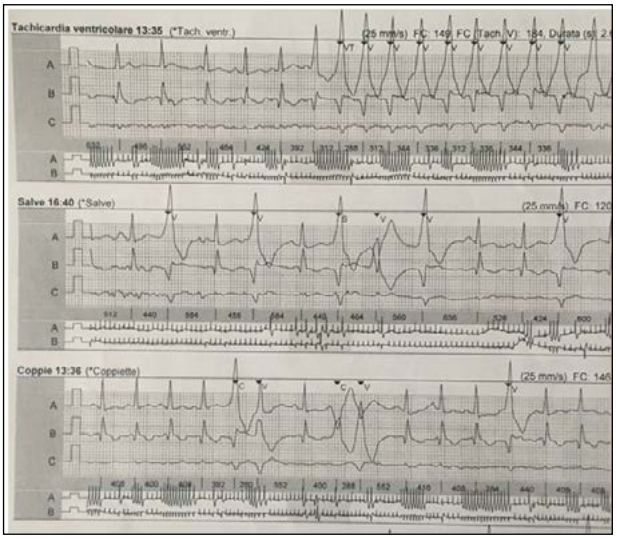

Figure 1. NSVT at 24 hour Holter ECG recording.

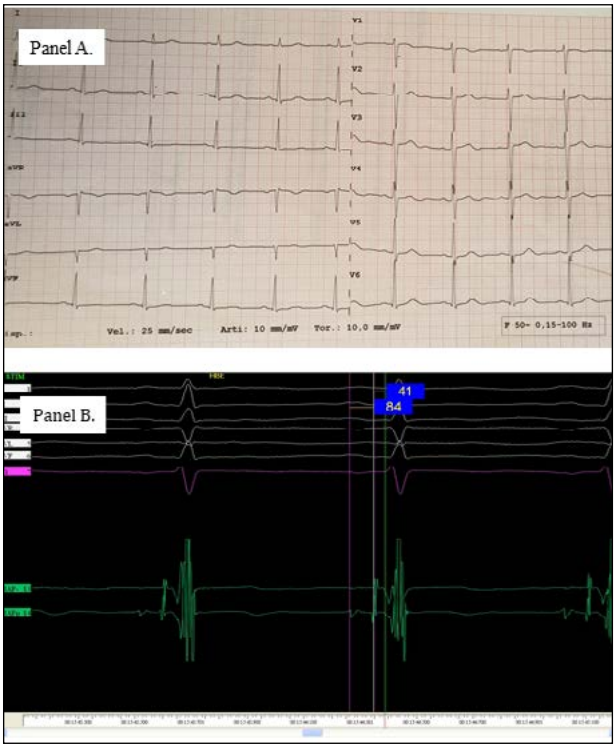

Figure 2. Panel A. basal ECG-Panel B. HV interval at electrophysiological study.

nosed with DM1 three years earlier showing an increased CTG repeat length, with a number size defined between 50-500. The patient showed temporal muscle atrophy, proximal weakness at lower limbs and grip and evoked myotonia. Needle electromyography (EMG) showed mild myopathic changes and myotonic discharges. The patient reported a case of SD in her family history (maternal grandmother, at age 29). On arrival at our department the vital signs were the following: blood pressure 110/70 mmHg, peripheral oxygen saturation 99% in room air, heart rate 70 bpm. No specific drugs were assumed. Basal surface ECG showed sinus rhythm with a PR interval of 0.16 seconds and a QRS duration of 0.10 seconds. A recent 24 hours-Holter ECG monitoring recorded 27 episodes of non-sustained VTs (Fig. 1). Echocardiography showed preserved left ventricular (LV) systolic and diastolic function (EF 55%). Cardiac magnetic resonance (CMR) excluded intramyocardial fibrosis and confirmed a preserved biventricular systolic function. Electrophysiological study (EPS) showed normal correct sinus node recovery time (CSNRT 420 msec) and atrio-ventricular node conduction times (AH 84 msec; HV 41 msec) (Fig. 2). During ventricular stimulation from right ventricle apex (refractory period: 200 msec with drive of 600 msec), sustained ventricular fibrillation (VF) was easily induced (coupling intervals: 600-240-200 msec) and rapidly interrupted with single external DC-Shock. EPS was followed by dual chamber transvenous ICD implantation. ICD remote home monitoring (Medtronic CareLink® System) was provided for VTAs burden surveillance. Low dosage of bisoprolol was started with rapid relief from palpitation and dizziness. ACE-inhibitors were not started for hypotension. Patient was regularly discharged on 7th day from admission. At 1 year of follow up, the patient was still asymptomatic on bisoprolol, and in good clinical conditions. Remote monitoring showed VTAs monthly burden of 0.4%.

Discussion

Myotonic Dystrophy type 1 is an autosomal, dominant disorder due to CTG expansion in the untranslated 3' region of the DM1 protein kinase (DMPK) gene and is the most frequent muscular dystrophy in adults [1]. Cardiac involvement often precedes the muscular/neurological signs and up to one third of deaths is sudden and unexpected, showing that risk stratification is crucial in the management of DM1 [2]. SCD is most likely due to high-degree atrioventricular (AV) block; however, ventricular tachyarrhythmias are increasingly recognised as a common finding in these patients and might explain some cases of sudden death after pacemaker (PM) implantation [5]. Paroxysmal supraventricular tachyarrhythmias (atrial fibrillation, atrial flutter, atrial tachycardia) are a common finding on electrocardiographic monitoring with a prevalence up to 25% in DM1 patients. Atrial fibrillation/flutter (AF/AFl), a frequent feature in DM1 patients, may be the first clinical manifestation of the disease in

young patients and seems to increase the mortality in this population.[6] Given the high risk of supraventricular arrhythmias and their consequences, clinical and instrumental strategies for reducing the risk of atrial fibrillation are of pivotal importance in the optimization of clinical management.

Previous studies showed that abnormalities of the conduction system on surface ECG (PR interval > 240 msec, QRS interval > 120 msec, left bundle branch block) were independent risk factors for SD, presumably owing to the progression of conduction system disease to a complete atrio-ventricular block [5]. Currently, a HV interval > 70 msec at EPS is predictive of an appropriate indication for PM implantation in DM1 [7]. However, a significant number of SD is observed, despite the PM implantation, and some clinicians consider ICD as the preferential device for DM1 patients with conduction disease [8,9]. Myocardial fibrosis at CMR is present in 40% of DM1 patients and is not predicted by ECG, ECG-Holter monitoring and echocardiography, but is often associated with increased risk of SD [10]. According to the latest guidelines, the prophylactic implantation of ICD in patients with neuromuscular disorders should follow the same criteria as in non-ischemic dilated cardiomyopathy, so an ICD implantation may be reasonable in DM1 patients when pacing is needed [2]. However, in DM1, VTAs may occur even in patients with normal ECG and preserved LV systolic function. A recent large study on 1388 DM1 patients reported a 3.6% cumulative incidence of SD over a median 10-year follow-up, with the involvement of multiple mechanisms including conduction defects, sustained VTAs and extracardiac causes [4]. According to this study, age, male sex, syncope, heart rate and 1st degree AV block were independent predictors of overall mortality, at a multivariate analysis. Of note, age, family history of SD and left bundle brunch block were significantly associated with SD. Furthermore, non-sustained VTAs, recorded at Holter ECG monitoring, were the only predictors of sustained VTAs [4].

We describe the case of a young woman affected by early stage DM1 – with preserved cardiac function and absence of conduction abnormalities either at basal surface ECG or EPS evaluation – who needed ICD implantation for the presence of syncope, non-sustained VTAs at 24-Hour ECG monitoring, and a family history of SD associated with a VF inducibility at EPS. The decision for ICD implantation was in accordance with ESC Syncope Guidelines. The high ventricular vulnerability was not associated with intramyocardial scar or cardiac dysfunction at CMR. In our view, further studies are needed and a revision of current recommendations for ICD implantation in DM1 could be considered, mostly in patients presenting with syncope, non-sustained VTAs and a family history of SD. In this subset of patients, the evidence of high ventricular vulnerability at EPS, rather than cardiac dysfunction or conduction abnormalities, would be useful in identifying subjects eligible for ICD implantation to prevent unexpected deaths, whose incidence is not negligible. In conclusion, to date, the best strategy for SD risk stratification in DM1 patients is not yet well known. In addition to Groh criteria (PR ≥ 240 ms, QRS ≥ 120 ms or atrial tachy-arrhythmias), recent evidences showed that age, syncope, family history of SCD and left bundle branch block are independent predictors of SD. Nevertheless, the role of ICD implantation in DM1 patients with preserved systolic function is not fully clarified and the choice of best device to implant is based on patient-centred electrophysiological evaluation. Our clinical case confirms the recommendations that family history of SD, syncope with palpitation and non-sustained VTAs may be considered red flags for high risk of SD in DM1 patients, even when conduction disorders, ventricular dysfunction and CMR ventricular fibrosis cannot be detected with conventional instrumental investigation. This risk is confirmed by the high ventricular vulnerability at the EPS evaluation in these patients. Further studies are needed to evaluate if the EPS-guided therapy, including the prophylactic ICD implantation in inducible patients, will prevent SD in DM1 patients without ventricular dysfunction and conduction disorders compared with conventional therapy.

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Neuromuscular tetanic hyperexcitability syndrome associated to a heterozygous *Kv1.1 N255D* mutation with normal serum magnesium levels

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Mutations of the main voltage-gated K channel members Kv1.1 are linked to several clinical conditions, such as periodic ataxia type 1, myokymia and seizure disorders. Due to their role in active magnesium reabsorption through the renal distal convoluted tubule segment, mutations in the *KCNA1* gene encoding for Kv1.1 has been associated with hypomagnesemia with myokymia and tetanic crises. Here we describe a case of a young female patient who came to our attention for a history of muscular spasms, tetanic episodes and muscle weakness, initially misdiagnosed for fibromyalgia. After a genetic screening she was found to be carrier of the c.736A > G (p.Asn255Asp) mutation in *KCNA1*, previously described in a family with autosomal dominant hypomagnesemia with muscular spasms, myokymia and tetanic episodes. However, our patient has always presented normal serum and urinary magnesium values, whereas she was affected by hypocalcemia. Calcium supplementation gave only partial clinical benefit, with an improvement on tetanic episodes yet without a clinical remission of her spasms, whereas magnesium supplementation worsened her muscular symptomatology.

Key words: hypomagnesemia, tetany, *KCNA1*

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Introduction

Voltage-gated K channels are a family of membrane proteins involved in determining the potential of the cellular membrane at rest, shaping action potentials and controlling neuronal excitability [1]. In these proteins, a central ion conduction channel is encircled by four subunits, each consisting of six transmembrane-spanning-helices (S1-S6) where segments S1-S4 form the voltage-sensing domain and segments S5-S6 form the pore domain [2]. Among voltage-gated K channels, the Shaker-related group Kv1.1 is encoded by the *KCNA1* gene and is abundantly expressed in excitable and non-excitable cells. Its mutations may cause both a significant reduction in current amplitude and altered kinetic properties related to K+ balance [1]. So far, mutations in Kv1.1 have been linked to several disorders such as periodic episodic ataxia type 1 (EA1), presumably caused by defective Kv1.1 in the cerebellum [3], myokymia, caused by the alteration of Ca2+ homeostasis due to the dysfunction of juxtaparanodal Kv1.1 channels [4], and isolated neuromyotonia [5]. The wide expression of Kv1.1 in the nervous system explains the manifold clinical phenotypes of

its mutations [6]. The molecular genetic testing of *KCNA1* has further broadened the clinical spectrum of the disorders associated to mutations in the gene, *e.g.* delay in motor development, choreoathetosis, cognitive dysfunction, transient postural abnormalities in infancy, shortening of the Achilles tendon in children [6], non-ataxic cataplexy [7] and atypical episodic ataxia with migraine and hyperthermia [8].

Finally, and interestingly, due to the Kv1.1 localization in the renal distal convoluted tubule segments, mutations in *KCNA1* have also been related to hypomagnesemia leading to tetany and myokymia, as shown in a single family[3]. In kidney, the K+ secretion via Kv1.1 provides an electrical gradient that drives Mg2+ reabsorption via the permeable transient receptor potential cation channel TRPM6. The amino acid substitution of a highly conserved asparagine for an aspartic acid in the third transmembrane segment of Kv1.1 (p.Asn255Asp) results in a non-functional channel causing an autosomal dominant hypomagnesemia associated to muscle cramps, tetanic episodes, tremor, and muscle weakness[3]. The substitution of the asparagine with other hydrophobic, polar, or charged amino acids cause a nonfunctional channel, confirming *in vitro* the central role of asparagine in the right functioning of the channel[1]. An additional *KCNA1* mutation resulting in a nonfunctional channel (p.Leu328Val) with hypomagnesemia has been reported in a young female patient affected by tetany who had an abnormal urinary Mg2+ excretion[2]. This finding suggested a possible role of *KCNA1* mutations besides p.Asn255Asp in hypomagnesemia.

Notwithstanding the large variability of symptoms in EA1, the most common phenotype associated to *KCNA1* mutations, the penetrance of the hypomagnesemia phenotype has yet not been assessed, as it has rarely been reported in EA1 [5]. Here we describe a case of muscular spasms and tetanic episodes in a female patient affected by Kv1.1 N255D mutation with normal serum magnesium levels.

Case report

A 31 years old female patient came to our attention in 2012 at the Neuromuscular Unit in Santa Chiara Hospital, Pisa- Italy, for a history of recurrent muscular spasms, tetanic episodes, diffuse and persistent muscle weakness and diarrhea. The muscular spasms were mainly localized at face and hands, lasted from several minutes to one hour, were often associated to tachycardia and worsened with carbohydrates ingestion and physical effort. These symptoms started in infancy with low frequency and low intensity but exacerbated after her first delivery and during breastfeeding, so that she required a medical consult.

The patient had no track record of neuromuscular diseases in her family, except for a history of occasional muscular spasms in her father and a juvenile cardiac disease with heart conductance alterations in her grandmother, not better investigated. After an initial misdiagnose of a rheumatological disorder (fibromyalgia), the patient was asked for a neurological evaluation and thus came to our attention. The neurological examination was normal, except for a mild and fluctuating weakness of iliopsoas muscles (MRC 4/5 bilaterally). She had no signs of myotonia or dyskinetic movement, neither she had ataxia. Objective clinical signs of cerebellar dysfunction, including nystagmus, multistep or overshoot saccades, dysmetria in the finger-nose test, and decomposition of movement of the legs on heel-to-shin test, were absent.

The patient underwent an electromyography (EMG) showing polyphasic motor unit potentials suggestive for myopathic changes; the EMG tetany test resulted positive in several occasions, as after 1'30" of physical effort, grouped motor unit discharges appeared and persisted for over 2 minutes. All biochemical values were normal, including blood count, renal function, hepatic function, thyroid function, estroprogestinic hormones, parathormone, glucose metabolism, autoimmune tests, GAD antibodies, and iron balance. The blood dosage of aldolases, creatine phosphokinase and the lactic dehydrogenase also resulted normal. Due to a reduced ammonium production in the ischaemic lactate-ammonia test, the patient underwent a DNA analysis for myoadenylate deaminase deficiency, resulting negative. Since the history of cardiac disease in her grandmother, DNA analyses for *LMNA* gene was also performed, resulting negative. Based on the muscular transient weakness, serum acetylcholine receptors antibodies (Ab) and muscle-specific tyrosine kinase Ab were tested, also resulting negative. Muscular spams coupled with tetanic episodes suggested to dose blood electrolytes, discovering a mild hypocalcemia (ionized calcium 3.3 mg/dl with normal values of 4.4-4.9 mg/dl), while the magnesium dosage in the serum always resulted normal, although at lower limits.

The clinical picture therefore prompt us to perform genetic analysis for channelopathies. The NGS analysis showed a heterozygous mutation c.736A > G (p.Asn255Asp) in *KCNA1*, further confirmed with Sanger method, previously reported in association with autosomal dominant hypomagnesemia with sudden episodes of facial myokymia, tremor, muscle spasms with painful cramps, muscular weakness, and tetanic contraction episodes. A genetic test for mutations in exon 1 of *KCNA1* on both parents was conducted through PCR amplification and direct sequencing, but resulted negative. This at first instance suggests that the patient's *KCNA1* mutation appeared *de novo* [2]. The patient completed the analysis

with urinary magnesium excretion that showed a normal magnesium excretion (81,5 mg/24h with normal values less than 120). Following literature reports, a magnesium integration was added to her treatment, but was later discontinued due to a subjective worsening of the muscular symptoms. The same adverse effects were reported for a potassium supplementation. Several myorelaxants (*e.g.* baclofen, benzodiazepines, cyclobenzaprine, and tizanidine) and neuromodulator drugs (*e.g.* trazodone, pregabalin, and amitriptyline) were tested, but only calcium integration had a slight clinical benefit on the tetanic episodes and on the muscular weakness, whilst muscular cramps and spasms persisted. Eventually, a cerebral MRI and a cardiological balance with electrocardiogram, echocardiogram and troponine dosage all resulted normal. The patient is currently monitored on a yearly basis and appears in a stable condition.

Discussion

Several genes are known to be involved in hereditary hypomagnesemia, including tight junction proteins claudin 16 and 19, the thiazide-sensitive sodium chloride cotransporter (NCC), the γ-subunit of the Na+/K+ ATPase (FXYD2), TRPM6, and the magnesiotropic hormone EGF [10]. The c.736A > G (p.Asn255Asp) mutation in *KCNA1* has been reported to cause an autosomal dominant hypomagnesemia with sudden episodes of facial myokymia, tremor, muscle spasms with painful cramps, muscular weakness, and tetanic contraction episodes [3]. This is to be put in relation with the localization of Kv1.1 in the apical membrane of distal convoluted tubule (DCT) cells, where TRPM6 controls Mg2+ entry driven by its electrochemical potential. Mutations in Kv1.1 protein, while having no direct effect on TRPM6, exhibit reduced K+ conductance, thus depolarizing the apical membrane of DCT cells reducing the electrical driving force, favoring Mg2+ entry and leading to renal Mg2+ loss [9].

Puzzling, hypomagnesemia has rarely been reported in relation to *KCNA1* mutations. In the family described by Glaudemans et al. [3], the severity of the phenotype varied among affected members, with some cases of severe tetanic crises (in one case leading to death in infancy) and other cases having a milder muscular involvement. Low serum Mg2+ levels were observed in variable amounts among family members, whereas urinary Mg2+ excretion levels were normal, suggesting an impaired tubular Mg2+ reabsorption. In the patients described, both serum K+ and Ca2+ levels and urinary Ca2+ excretion levels were normal, in contrast with other forms of inherited hypomagnesemia [10]. In these patients, magnesium integration led to partial clinical benefit, yet without a complete control on the muscular symptoms.

In the case here reported, the clinical phenotype concerning the muscular involvement was similar to that reported in previous literature. However, the patients' serum and urinary magnesium dosages resulted normal in repeated measures. Interestingly, low values of serum ionized calcium were consistently reported, and calcium supplementation was indeed the only treatment that resulted effective in treating the symptoms, while magnesium integration resulted ineffective and perhaps worsened the symptoms, in contrast to what described in Glaudemans et al. [3]. These findings are in accordance with a key role in DCT cells in ionic balance not only for Mg2+, but also for Na+, K+, and Ca2+. The presence of other channels co-expressed throughout the distal tubule, *e.g.* ROMK for K+ and ENac for Na+, makes it so that Kv1.1 is not the only contributing to apical conductance, underlying the complexity of the ionic balance system *in vivo* [9]. Altogether, our findings suggest to further research efforts in the characterization of the role of *KCNA1* mutations hypomagnesemia and the related clinical treatment.

Acknowledgements

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NEWS FROM AROUND THE WORLD

AIM

The Italian Association of Myology (AIM) sponsored, between January and March 2020, two congresses that have been moved to later date due to the COVID 19. The first one should have been held in Pisa, the 20-21 March, entitled *E-health & Innovation to overcome barriers in Neuromuscular Diseases* and the second in Messina, the 4th April, named *Muscle Diseases in the reality of the territory: the importance of early diagnosis*.

Many activities of the Association during this period have been dedicated to the organization of the next joint congress of the Italian Association of Myology (AIM) and the Italian Association for the Study of the Peripheral Nerve System (ASNP) that will be held in Matera, 3-6 June 2020, emergency covid-19 permitting. The preliminary program can be found at the end of section Forthcoming Meetings of this issue (see p. 41). Further information can be find at the following link:// www.miologia.org/news/congresso-congiunto-aim-asnp

Carmelo Rodolico
AIM Secretary

MSM

The 14th Meeting of the Mediterranean Society of Myology (MSM) the meeting is moved to spring 2021. Proposals to organize and host the event are welcome.

WMS

This year marks the Jubilee 25th International Annual Congress of the World Muscle Society that will take place 30th September – 4th October 2020, in Halifax, Nova Scotia, Canada. The congress venue is the new Halifax Convention Centre at 1650 Argyle Street, in the heart of this Atlantic seaport. The opening reception will be held on Wednesday 30th September 2020 in the Canadian Museum of Immigration at Pier 21, with the networking dinner held on Saturday 3rd October at the magnificent Halifax Citadel.

The academic and clinical teams from the Hospital for Sick Children and University of Toronto led by Jim Dowling and Jiri Vajsar, together with the Local Organising Committee and the WMS Programme Committee, are the hosts and organisers of this meeting. The 4-day meeting will be an opportunity to catch up on the latest developments in neuromuscular diseases from around the world.

Following the longstanding tradition of the WMS, the meeting will be preceded by a teaching course, on 29-30th September 2020, at the IWK Health Centre in Halifax.

Contributions about new advances across the neuromuscular field are very welcome. The main thematic topics that will be addressed in the plenary sessions are:

1. New developments in congenital muscle disease;
2. Gene modifiers and gene delivery in neuromuscular disorders;
3. Advances in the treatment of neuromuscular disorders.

FORTHCOMING MEETINGS

2020

February 5-7

2nd International Scientific & Clinical Congress on Spinal Muscular Atrophy. SMA Europe-Evry, France. Information: website: *https://evry2020.sma-europe.eu*

March 6-8

Developing best practice guidelines for management of mouthpiece ventilation in neuromuscular disorders. 252nd ENMC Workshop. Information: website: *https://www.enmc.org/*

March 11-14

8th Dysferlin conference, Jain Foundation. Orlando, Florida, USA. Information: website: *https://www.jain-foundation.org*

March 16-17

International Conference on Orphan Drugs & Rare Diseases. Berlin, G. Information: website: *https://www.meetingsint.com/conferences/orphandrugs-raredisease*

March 20-22

Skeletal muscle laminopathies – natural history and clinical trial readiness. 253rd ENMC Workshop. Information: website: *https://www.enmc.org*

April 25 - May 1

72nd Annual Meeting American Academy of Neurology, Toronto, Ontario, Canada. Information: website: *https://www.aan.com*

June 3-6

XX Congresso Nazionale AIM. Matera, IT. Information: website: *https://www.miologia.org*

June 6-9

The European Human Genetics Conference. Berlin, Germany. Information: website: *https:// www.eshg.org*

July 6-9

New directions in Biology and Disease of Skeletal Muscle Conference, New York, NY, US.
Information: website: *https://myology.institute.ufl.edu/conferences/new-directions*

September 25-29

Muscle Study Group Annual Scientific Meeting, Washington, US. Information: website: *https://musclestudygroup.org/events/2020-annual-meeting*

September 30 - October 4

25th Congress of World Muscle Society. Halifax. Toronto, Canada. Information: website: *www.worldmusclesociety.org*

October 27-31

ASHG Annual Meeting. San Diego, CA,USA. Information: website: *www.ashg.org*

2021

June 12-15

The European Human Genetics Conference. Glasgow, United Kingdom. Information: website: *https://www.eshg.org*

September 21-25

26th Congress of World Muscle Society. Prague, Czech Republic. Information: website: *www.worldmusclesociety.org*

October 19-23

ASHG Annual Meeting. Montreal, Canada. Information: website: *www.ashg.org*

Associazione Italiana
Sistema Nervoso Periferico

Congresso Congiunto
AIM - ASNP

MATERA
3 - 6 Giugno 2020

Auditorium Raffaele Gervasio

XX Congresso Nazionale AIM
Associazione Italiana di Miologia

X Congresso Annuale ASNP
Associazione Italiana per lo studio
del Sistema Nervoso Periferico

Presidenti del Congresso
Prof. Gian Maria Fabrizi - ASNP
Prof. Carlo Minetti - AIM

Presidente del Comitato Scientifico
Prof. Antonio Toscano

Presidente del Comitato Organizzatore Locale
Dott. Pietro Masciandaro

Preliminary Programme

Congresso Congiunto

AIM - ASNP

XX Congresso Nazionale AIM

Associazione Italiana di Miologia

X Congresso Annuale ASNP

Associazione Italiana per lo studio
del Sistema Nervoso Periferico

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Dott. Pietro Masciandaro

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Carmelo Rodolico
Marcello Calogero Romano
Angelo Schenone
Gabriele Siciliano

MATERA

3 - 6 GIUGNO 2020

Auditorium Comunale
Raffaele Gervasio

Associazione Italiana
Sistema Nervoso Periferico

Congresso Congiunto
AIM - ASNP

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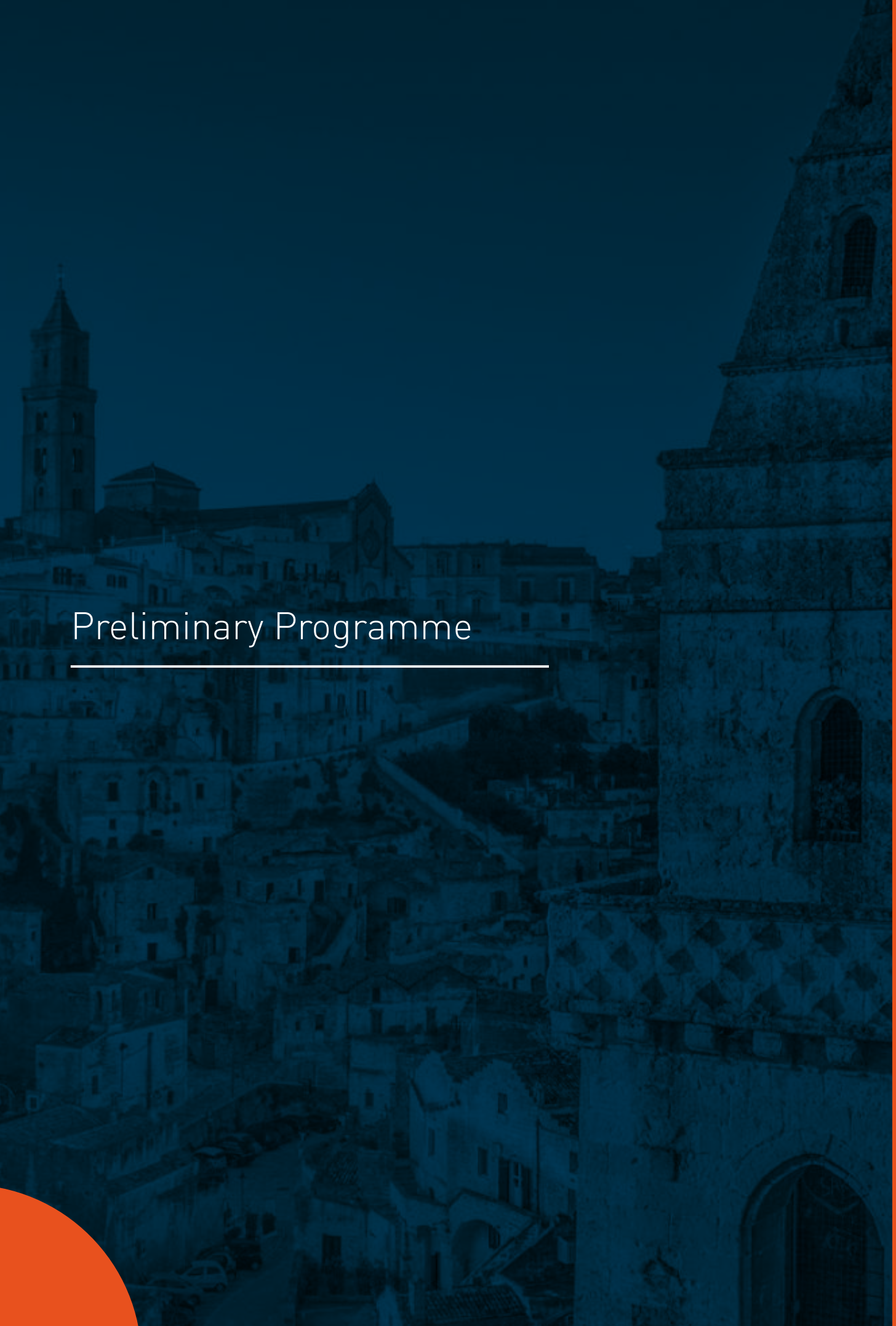

Preliminary Programme

Mercoledì 3 Giugno

12.00 - 14.00 | **Workshop:**
Innovative therapeutic strategies in genetic neuromuscular diseases (Part I)
- An "antisense" approach for neuromuscular diseases | *A. McCampbell*
- Gene therapy for SMA: translating research into clinical reality | *M. Bernat Fuertes*
- In vivo gene therapy for Pompe disease | *F. Mingozzi*
- nmDMD: from clinical trials to clinical practice | *M. Pane*

14.00 - 14.30 | Greetings and introduction

14.30 - 16.00 | **Workshop:**
New tools in the management of patients with neuromuscular disorders
- Genetic modifiers as a tool for personalized treatment of neuromuscular disorders *L. Bello*
- Animal models as a tool to design novel therapeutical strategies | *S. Previtali*
- Computation Neurology: devices, data and models for characterization and modelling of neuromuscular diseases | V. *Sanguineti*
- Digital health solutions to increase sensitivity of outcome measures in clinical and trial settings in neuromuscular disorders | *S. Messina*

16.00 - 17.00 | **Oral communications**

17.00 - 17.30 | **Coffee break**

17.30 - 18.00 | **Lecture: Neuromuscular disorders prevention: newborn screening, prenatal and pre-implantation diagnosis and counseling** | *L. Stuppia*

18.00 - 20.00 | **Workshop:**
Autophagy and other pathogenic mechanisms in NMDs
- IGF-1 mediated signalling to counteract muscle atrophy | *A. Musarò*
- New insights of autophagy regulation and involvement in muscle to neurons communication | *M. Sandri*
- Autophagy in peripheral neuropathies: mechanisms and treatment options *V. Timmerman*
- When glycogen becomes insoluble: clinical features and pathobiology of polyglucosan storage disorders | *A. Oldfors*

20.00 - 20.45 | **New treatment horizons in the management of SMA**
- Recent scientific evidences in the context of early onset SMA | *R. Masson*
- What's new in the landscape of later onset SMA? | *M.C. Pera*

20.45 | **Welcome reception**

Giovedì 4 Giugno

07.30 - 08.30 | **Breakfast Seminar:**
Transthyretin-related Amyloidosis
- Molecular mechanisms | L. *Obici*
- Clinical presentation, diagnosis and treatment of peripheral neuropathy | *G. Vita*
- Clinical and therapeutic aspects of cardiomyopathy | *C. Rapezzi*

08.30 - 10.00 | **Oral communications**

10.00 - 10.30 | **Lecture:** "**High cost technologies and economic sustainability of the Italian National Health Service. How long can we afford it for?**
R. Tarricone

10.30 - 10.50 | **Coffee break**

10.50 -11.50 | **Workshop:**
Imaging techniques in muscle and peripheral nerve diseases
- Ultrasound as a multi-tool for Muscle and Nerve | *L. Padua*
- MRI in peripheral nerve disorders | *S. Gerevini*
- Imaging vs morphology in the current diagnostic workup of muscle disorders
M. Garibaldi

11.50 -13.05 | **Oral communications**

13.05 -16.00 | **Lunch and poster viewing**

16.00 - 17.30 | **Oral communications**

17.30 - 18.50 | **Workshop:**
Innovative diagnostic aspects in neuromuscular disorders
- New histopathological markers for the diagnosis of the myopathies: the vintage that is updated | *C. Fiorillo*
- Skin biopsy: beyond the intraepidermal nerve fiber (IENF) density | *M. Nolano*
- Genome analyses in muscular disorders | *V. Nigro*
- NGS in CMT-related disorders | *F. Taroni*

18.50 - 20.00 | **Assemblee AIM e ASNP**

Venerdì 5 Giugno

07.30 - 08.30 | **Breakfast seminar:**
"Respiratory dysfunction in patients with Duchenne Muscular Dystrophy: state of the art and new acquisitions"
- The effectiveness of idebenone in the treatment of DMD | *E. Mercuri*
- Why is idebenone effective on respiratory function? | *A. Vianello*

- Respiratory emergency management in DMD | *F. Racca*

08.30 - 09.30 | **Neuromuscular club**

09.30 - 11.10 | **Workshop:**
Innovative therapeutic strategies in genetic neuromuscular diseases (Part II)
- Innovative strategies therapeutic in Muscle dystrophies | *G. P. Comi*
- Advances in the treatment of hereditary ATTR amyloidosis | *A. Mazzeo*
- Challenges in treating CMT: do we see daylight? | *D. Pareyson*
- New Therapeutic approaches in SMA | *E. Mercuri*
- New therapeutic strategies in metabolic myopathies | *O. Musumeci*

11.10 - 11.30 | **Coffee break**

11.30 - 12.00 | **Lecture: Check point inhibitors: Immunopathology and advances in management** | *M. Dalakas*

12.00 - 13.00 | **Oral communications**

13.00 - 16.00 | **Lunch and poster viewing**

16.00 - 17.30 | **Round table:**
Medical doctors, Neuromuscular experts, Patients and Associations
GMN 4th edition: facts and perspectives

17.30 - 18.30 | **Workshop:**
New therapeutic frontiers in dysimmune diseases of muscle, NM junction and peripheral nerve disorders
- New therapeutic strategies for the treatment of myasthenia gravis | *A. Evoli*
- New immunomodulatory treatment for immune-mediated neuropathies | *L. Benedetti*
- State of the art on the therapy of autoimmune myopathies | *M. Mirabella*

18.30 - 20.00 | **Oral communications**

21.00 | **Social dinner**

Sabato 6 Giugno

07.30 - 08.30 | **Breakfast seminar:**
"New perspectives for Myotubular Myopaties"
- XLMTM: clinical spectrum, epidemiology, Nat-His MTM1 Study | *A. D'Amico*
- Histopathology of centronuclear myopathies | *C. Bruno*
- Gene therapy and Aspiro study update | *W. Müller-Felber*

Sabato 6 Giugno

08.30 - 10.00 | **Oral communications**

10.00 - 10.30 | **Coffee break**

10.30 - 11.30 | **Workshop:**
Paraneoplastic diseases of muscle, NM junction and peripheral nerve: diagnostics and therapy

- Paraneoplastic neuropathies | *S. Ferrari*
- Presynaptic paraneoplastic disorders of the neuromuscular junction: an update *R. Liguori*
- Paraneoplastic disorders of skeletal muscles | *C. Rodolico*

11.30 -12.45 | **Oral communications**

12.45 - 13.30 | **Awards and Conclusions**

Chiesa di San Francesco d'Assisi - Matera

INSTRUCTIONS FOR AUTHORS

Acta Myologica publishes articles related to research in and the practice of primary myopathies, cardiomyopathies and neuromyopathies, including observational studies, clinical trials, epidemiology, health services and outcomes studies, and advances in applied (translational) and basic research.
Manuscripts are examined by the editorial staff and usually evaluated by expert reviewers assigned by the editors. Both clinical and basic articles will also be subject to statistical review, when appropriate. Provisional or final acceptance is based on originality, scientific content, and topical balance of the journal. Decisions are communicated by email, generally within eight weeks. All rebuttals must be submitted in writing to the editorial office.

Starting from 2020, a publication fee of 200 Euros is required. The Corresponding Author must fill in the appropriate form and send it with the corrected proofs. 50% off is offered for members of Associazione Italiana di Miologia (AIM) and/or Mediterranean Society of Myology (MSM) in good standing with dues. A copy of the payment receipt for the current year is mandatory to prove the membership).

On-line submission
Manuscript submission must be effected on line: **www.actamyologica.it** according to the following categories:
Original articles (maximum 5000 words, 8 figures or tables). A structured abstract of no more than 250 words should be included.
Reviews, Editorials (maximum 4000 words for Reviews and 1600 words for Editorials). These are usually commissioned by the Editors. Before spontaneously writing an Editorial or Review, it is advisable to contact the Editor to make sure that an article on the same or similar topic is not already in preparation.
Case Reports, Scientific Letters (maximum 1500 words, 10 references, 3 figures or tables, maximum 4 authors). A summary of 150 words may be included.
Letters to the Editor (maximum 600 words, 5 references). Letters commenting upon papers published in the journal during the previous year or concerning news in the myologic, cardio-myologic or neuro-myologic field, will be welcome. All Authors must sign the letter.
Rapid Reports (maximum 400 words, 5 references, 2 figures or tables). A letter should be included explaining why the author considers the paper justifies rapid processing.
Lectura. Invited formal discourse as a method of instruction. The structure will be suggested by the Editor.
Congress Proceedings either in the form of Selected Abstracts or Proceedings will be taken into consideration.
Information concerning new books, congresses and symposia, will be published if conforming to the policy of the Journal.
The manuscripts should be arranged as follows: 1) Title, authors, address institution, address for correspondence; 2) Repeat title, abstract, key words; 3) Text; 4) References; 5) Legends; 6) Figures or tables. Pages should be numbered (title page as page 1).
Title page. Check that it represents the content of the paper and is not misleading. Also suggest a short running title.
Key words. Supply up to three key words. Wherever possible, use terms from Index Medicus – Medical Subject Headings.
Text. Only international SI units and symbols must be used in the text. Tables and figures should be cited in numerical order as first mentioned in the text. Patients must be identified by numbers not initials.
Illustrations. Figures should be sent in .jpeg or .tiff format. Legends should be typed double-spaced and numbered with Arabic numerals corresponding to the illustrations. When symbols, arrows, numbers, or letters are used to identify parts of the illustrations, each should be explained clearly in the legend. For photomicrographs, the internal scale markers should be defined and the methods of staining should be given.
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