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Long-term follow-up of patients with congenital myasthenic syndrome caused by *COLQ* mutations

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Abstract

Congenital myasthenic syndromes (CMS) are clinically and genetically heterogeneous inherited disorders characterized by impaired neuromuscular transmission. Mutations in the acetylcholinesterase (AChE) collagen-like tail subunit gene (*COlQ*) cause recessive forms of synaptic CMS with end plate AChE deficiency. We present data on 15 *COLQ* -mutant CMS carrying 16 different mutations (9 novel ones identified) followed-up for an average period of 10 years. The mean age at the first examination was 19 years old (range from 3 to 48). We report relapses during short or long-term periods characterized by worsening of muscle weakness sometimes associated with respiratory crises. All the relapses ended spontaneously or with 3–4 DAP or ephedrine with no residual impairment. The triggering factors identified were esterase inhibitors, effort, puberty or pregnancy highlighting the importance of hormonal factors. There was no genotype–phenotype correlation. At the end of the follow-up, 80% of patients were ambulant and 87% of patients had no respiratory trouble in spite of severe relapses.

Keywords: Congenital myasthenic syndrome; *COLQ*; Acetylcholinesterase; Relapse; Mutations

1. Introduction

Congenital myasthenic syndroms (CMS) are clinically and genetically heterogeneous inherited disorders in which the safety margin of neuromuscular transmission is compromised [1,2]. Fourteen CMS-related genes have been described so far, coding for proteins involved in neuromuscular transmission [2–6]. However, it is estimated that half of the CMS remain genetically unidentified. The syndromes are classified according to the localization of the corresponding defect at the neuromuscular junction as presynaptic, synaptic basal-lamina associated and postsynaptic [1,2].

Recessive synaptic CMS caused by acetylcholinesterase (AChE) deficiency were first described in 1977 [7]. Since then, few cases of partial or complete deficiency of the enzyme have been reported [8]. Absence of AChE in the synaptic space extends the lifetime of acetylcholine and

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leads to the synaptic basal-lamina associated CMS [9,10] by increasing the duration of the end-plate current [7]. This outlasts the refractory period of the muscle fibers and evokes a desynchronized compound muscle action potential (CMAP) following the electrical stimulation of the nerve-muscle complex with the appearance of a so-called 'double CMAP' [7]. The collagenic tail of endplate AChE or *COLQ* is composed of (i) a N-terminal proline-rich region attachment domain (PRAD) linked to the catalytic subunits, (ii) a triple helical homotrimeric collagen-like tail subunit structure, and (iii) a C-terminal region enriched in charged residues and cysteines allowing for the anchoring of the enzyme into the basal lamina. *COLQ* plays an important structural role at neuromuscular junctions by anchoring and accumulating AChE in the extracellular matrix [11]. Thirty different *COLQ* mutations have been described to date [12]. The majority of them are null mutations (frameshift, splice and nonsense mutations) which truncate the protein distally to PRAD [1,13,14]. The reported missense substitutions are located in the C-terminal region and potentially affect the insertion of the molecule in the membrane [4]. Clinically, patients harbouring *COLQ* mutations are affected from an early age with a progressive muscle weakness and a respiratory insufficiency. Associated slow pupillary light response, double CMAPs and no beneficial effect or even worsening of the muscle weakness after administration of AChE inhibitors are considered as diagnostic clues pointing to *COLQ*-related-CMS [14]. Here, we present the clinical and molecular genetics findings of 15 patients with *COLQ* mutations for an average period of 10 years. The long-term follow up of the considerable size of the group studied enables us to further describe the phenotypic and genotypic spectrum of end plate AChE deficiency. We observed a large clinical heterogeneity except the permanence of the double CMAP on EMG. Symptoms strongly fluctuated over time (including daily, weekly, monthly or even yearly changes). Worsening of muscle weakness was noticed during relapses in the short or long-term. Respiratory crises with bulbar signs were only present in relapses lasting for months or years. All the relapses ended either spontaneously or with 3–4 DAP or ephedrine without residual impairment. Triggering factors identified were hormonal, effort or esterase inhibitors.

2. Patients and methods

Fifteen patients (8 males, 7 females) from 14 unrelated families were followed up over a mean period of 10 years (range from 1 to 23 years). Patients 1 and 4 were previously reported [15]. The mean age at the first examination was 19 years old (range from 3 to 48). Clinical examinations were performed by neurologists [I.W., B.E. and T.S. at the Myology Institute of Pitié-Salpêtrière Hospital (13 patients), T.K. at Lausanne University Hospital (1 patient) and S.N. at Teheran University Hospital (1 patient)] during routine medical check-ups. Six patients (patients 2, 5, 9, 10–12 on Table 1a and b) were born from consanguineous marriages. Written informed consent was obtained from all patients or their legal representatives in accordance with the study protocol approved by the ethics committee of the Pitié-Salpétrière Hospital. All patients, but one, underwent motor nerve conduction studies performed on the ulnar, peroneal and facial nerves. Repetitive stimulation at 3 Hz was carried out on the ulnar, spinal, facial, radial and peroneal nerves and CMAPs were recorded on abductor digiti minimi, trapezius, orbicularis oculi, tibialis anterior and the anconeus muscles, respectively. Muscle biopsies were processed in 11 cases (patients 1–7, 11–13, 15) according to standard histological, histochemical, and immunohistochemical techniques [16–18]. Venous blood samples were obtained from the patients and their unaffected relatives. Genomic DNA was isolated using a blood DNA extraction kit according to the manufacturer's recommendations (Purification kit, Promega, Mannheim, Germany).

Identification of *COLQ* mutations was performed using direct sequencing on PCR products. All 17 exons and their flanking intronic regions were PCR amplified and sequenced using the Terminator v3.3 Cycle Sequencing Kit then run on the ABI3730 capillary electrophoresis system (Applied Biosystems). Electropherograms were analyzed using the SeqScape software (Applied Biosystem) and sequences were compared to reference sequences (NM_005677.3). The Alamut (www.interactive-biosoftware.com) software helped us to interpret the pathogenicity of variants found.

3. Results

3.1. Clinical phenotype

The clinical features of the patients are detailed in Table 1a (Fig. 1). The disease manifested at a mean age of 13 months (range from birth to 10 years). At birth, the only constant clinical sign was muscular hypotonia (5 cases). Other signs observed at birth included ptosis (4 cases) with or without ophthalmoparesis and bulbar signs as respiratory distress (2 cases) and dysphagia (3 cases). However, those clinical signs are not specific to birth since they have also been observed later on at the onset of the disease, and/or during relapses for bulbar signs. The age at which patients first walked was only delayed in 4 cases. During childhood, in all cases, proximal weakness muscle, affecting both upper and lower limbs, emerged and led to frequent falls. Additional clinical signs observed during childhood included hypoplasia of superior thorax and microtesticles (patients 8, 10), ankle contractures (patients 2, 3, 5), hip (patient 2) or knee (patient 15) retractions. During late childhood, scoliosis or hyperlordosis developed in 7 cases (patients 4, 6, 7, 10, 12–14). Arthrogryposis was not found in any of the patients. Pains, described as cramps or myalgia, were often reported and appeared to be triggered by exertion, the end of the day, cold winter or hot summer. Dysaesthesia was reported in patient 15.

Table 1a
Clinical data of the 15 patients with *COLQ* mutation.

Patient	Gender	Ethnic origin	Age of onset (months)	Age of walking (months)	Ophthalmoparesis /Ptosis/FW/ Dysphagia	Proximal /axial/ distal/ neck weakness	SPRL	Follow up	Pains	Respiratory crises/NIV/ tracheotomy	Relapses over months/ years besides hormonal factors	Aggravation during puberty/ pregnancy or deliver	Clinical status at the end of the follow up: Walton scale [28]/ NIV
1	F	French	6	12	+/+/−/+	+/+/+/+	+	13	+	+/+/−	−/+;+;+	+/*	VIII/+
2	M	Turkish	120	13	−/−/−/−	+/+/+/+	−	7	−	−	−/−	+/*	I/−
3	F	French/ Spanish	Birth	16	−/−/−/−	+/-/+/+	−	13	+	−	−/−	+/+	I/−
4	F	French	Birth	24	+/+/+/+	+/+/+/+	+	23	+	−	−/+	+/*	I/−
5	F	French	36	15	−/−/−/+	+/−/−/+	−	9	+	−	+/+ ;+	+/+	I/−
6	M	Italian	10	10	−/−/−/−	+/+/−/−	−	12	+	−	−/+	+/*	II/−
7	F	Portuguese	Birth	15	+/+/+/+	+/+/+/+	+	7	+	+/+/+	+/+	+/+	I/−
8	M	Filipino	24	16	+/+/+/−	+/+ /+/ +	−	6	+	+/+/+	−/−	+/*	I/−
9	M	Kurdish	24	14	+/+/+/+	+/+/+/+	−	6	+	−	−/−	+/*	I/−
10	M	Turkish	birth	34	+/+/+/+	+/+/+/+	−	5	+	+/+/+	−/+	*/*	VI/+
11	M	Spanish	72	15	−/+/−/−	+/−/−/−	−	1	−	−	nd	+/*	nd
12	F	Moroccan	24	13	−/+/−/−	+/+/−/+	nd	1	+	−	−/+	+/*	VI/−
13**	F	French	24	24	−/−/−/−	+/+/−/+	−	20	+	+/+/−	−/+	+/+	I/−
14**	M	French	13	13	−/−/−/+	+/+/−/+	−	13	−	−	−/+	−/*	I/−
15	M	French	Birth	18	+/+/−/−	+/+/−/+	−	3	+	−	−/−	*/*	III/−

* Not applicable; Gardner-Medwin and Walton scales [28]: grade 0: normal; grade I: unable to run freely; grade II: difficulty walking on tiptoes; grade III: climbing stairs with banister; grade IV: presence of Gowers' sign; grade V: unable to rise from the floor; grade VI: unable to climb stairs; grade VII: unable to rise from a chair; grade VIII: unable to walk without assistance; grade IX: unable to sit, drink or eat without assistance.
** Brother and sister. FW: facial weakness; SPRL: slow pupillary response to light; nd: no data; NIV: non invasive ventilation.

3.2. Time course of the disease

We observed both short- and long-term clinical fluctuations from our cohort.

3.2.1. "*Short-term fluctuations*": day or week

All patients experienced day-time fluctuations of the symptoms highly dependent on exercise. Patient 6 experienced two episodes of tetraparesis during one hour after rambling at the age of 17. Patient 2 could hardly move his mouth after having chewed gum all day long at the age of 18. All patients reported worsening of muscle weakness at the end of the day and/or over a 2 or 3 days period. In 5 cases, cold temperatures exacerbated weakness (patients 3, 4, 6, 10, 12). Heat with humidity had the same effect in 4 cases (patients 6, 7, 13, 15). All women, but one (patient 13), reported worsening of their symptoms during either premenstrual period, menstruations or even after the end of the cycle.

In addition to day-time fluctuations observed in the whole cohort, 3 cases (patients 2, 3, 10) exhibited muscle weakness that lasted for several weeks.

However, these short-term fluctuations (including days and weeks' ones), did not require any therapeutic treatment in any of the patients.

3.2.2. "*Long-term fluctuations*": month or year

All women of our cohort who had pregnancy triggered relapses whatever the way of delivery (eutocia or caesarean section). These relapses started during the last months of pregnancy and/or the post-partum phase (starting 5 to 10 days after the delivery), lasted from 3 to 8 months and recovered spontaneously without treatment, except for patient 7. In all patients, relapses were characterized by worsened weakness affecting the lower limbs. In two cases (patients 5 and 7), this muscular impairment was extremely severe, requiring the use of a crutch or even a wheelchair, and was associated with dysphagia. In addition, patient 7 suffered an acute respiratory distress leading to assisted ventilation and tracheotomy. The introduction of ephedrine allowed her to recover from this relapse without residual impairment.

One or several relapses over a 2 or 3 years period, characterized by dysphagia, dyspnoea or worsening of muscle weakness, have been noticed in 9 cases (patients 1, 4–6, 9, 11–14). Intense effort (patient 6) and esterase inhibitors in 2 cases (patients 1, 7) were the triggering factors identified. Two patients (patients 1, 7) were bedridden during 5–12 months, during such relapses. Tracheotomy, assisted ventilation and gastrostomy were necessary in patient 10.

Fig. 1. Patients with Col Q mutations. AB: Patient 1. At the age of 48 she can climb stairs using a bannister before progressive worsening. She is actually severely affected. She is unable to walk without assistance and needs non invasive ventilation. CD: Patient 5. At the age of 33 she is unable to run freely between relapses. She is actually mildly affected.

An increase in fatigability and muscle weakness lasting on average 5 years has been observed in the whole cohort around puberty (except for patients 10 and 15 who were followed up before puberty). Symptoms spontaneously ended at a mean age of 18 years without any medication. A wheelchair was necessary in one case (patient 12). No bulbar sign or acute respiratory distress ever occurred.

3.2.3. "Progressive worsening"

In patient 1, after relapses reported until the age of 48 years old (see above), the disease progressed gradually over 10 years characterized by a worsening of muscle weakness requiring a wheelchair, and dyspnoea needing assisted ventilation at home. When ephedrine was initiated, dysphagia and dysphonia resolved completely. Assisted ventilation was necessary for 12 h instead of 14 h a day and a crutch was adequate for mobility indoors.

3.2.4. Diagnostic delay

Diagnostic delay was delayed in the whole cohort up to a mean age of 23 years (range from 3 to 48 years) after symptom onset. Three different aetiologies have been suggested as follows:

(i) Seronegative auto-immune myasthenia was suggested in 6 cases (patients 2, 6–9, 15). Mean symptom onset was 30 months (from birth to 120 months). Muscle biopsy, when it was performed, was considered normal in 3 cases (patients 2, 6, 7). Steroids tried in 2 cases (patients 7, 9) were stopped after 3 months because of further impairment of symptoms.
(ii) Metabolic myopathy was the initial diagnosis in 4 cases (patients 1, 3, 4, 13). Mean symptom onset was 7 months (from birth to 24 months). Treatment with L-Carnitine and Coenzyme Q10 was given for 2 to 16 years. A subjective effect was found in 2 cases (patients 3, 4). Muscle biopsy showed lipid accumulation in 2 cases (patients 3, 13).
(iii) Congenital myopathy was the first diagnosis in 2 cases (patients 10, 12). Mean symptom onset was 12 months (birth to 24 months). Severe symptoms were observed: assisted ventilation, tracheostomy and gastrostomy at the age of 6 months in patient 10, whereas a wheelchair was required from age 10 to 17 years in patient 12.

3.2.5. Response to treatment

Acetylcholinesterase inhibitor treatment exacerbated symptoms or was ineffective in 11 cases. It potentially triggered severe relapses in 2 cases (patients 1, 7).

3, 4-Diaminopyridine (DAP) prescribed in 8 cases led to an improvement of the muscle weakness in 3 cases with 30–60 mg daily.

Since ephedrine is not widely available in France, this was only administered in 3 cases where it had remarkable benefits, improving muscle weakness in all cases and allowing one case to be weaned off ventilation (patient 7). Fluoxetine, administrated in one patient (patient 8), improved both fatigability and muscle weakness. Hormonal contraception prevented the deterioration of symptoms during menstruation in patient 4 whereas an improvement of the muscle weakness was noticed with menopause in patient 3.

3.2.6. Disease progression

At the end of follow-up, walking was possible without aids in 11 cases. An assistance or a crutch in 2 cases and a wheelchair in another case were reported. Assisted ventilation was required in 2 cases (12 h daily for patient 1 and 20 min daily for patient 10). Complementary nocturnal feeding by gastrostomy was necessary in one case (patient 10).

3.2.7. Laboratory, electromyography and muscle biopsy studies

Creatine kinase level was normal in all individuals tested. Antibodies against Ach receptors or against muscle-specific receptor-tyrosine kinase (MuSK) were negative in the serum.

EMG was carried out in 14 cases. A decremental (>10%) response following 3 Hz repetitive nerve stimulation was found in 95% of the 40 tested muscles in the whole cohort. The mean decremental response on ulnar nerve was 20% (range: 10–36%) recorded in 12 cases. But the most significant decremental response was obtained by stimulating the radial nerve (mean value 52%, range: 24–77%) in 6 cases. A double CMAP of abductor digiti minimi was also found in all patients after single stimulation of the ulnar nerve. Needle electromyography showed a myogenic pattern without spontaneous activity.

Muscle biopsy (Table 1b) was performed in 11 cases. It was first considered as normal in 5 cases leading to a second biopsy in 3 cases. Predominance of type I fibers and atrophy of type II fibers was reported in 7 cases. Predominance of type I fibers with lipid accumulation in type II fibers was observed in 2 cases (Fig. 2). In 3 cases, the AChE activity was studied and was abnormally low, whereas the density of receptors appeared normal. In addition, the end plates were much smaller than those in control muscles. Silver impregnation of nerve terminals on muscle sections showed a normal aspect of the terminal-

Table 1b
Biopsy and mutation results of the 15 patients.

Patient	Muscle biopsy	AChE at the end plates	Mutation (protein and cDNA level)	Consequence of mutations	Treatment by acetylcholinesterase inhibitors/ 3–4 DAP/ ephedrine
1	N/Predominance of type I fibers and atrophy of type II fibers	ND	c.107−1G>A/p.Arg236X (c.706C>T).	Splice mutation Non sense mutation	−/−/+
2	N	ND	p.Cys427Cys (c.1281C>T) Homozygote	Creation of a cryptic donor splice site in exon 16 leading to 19 exonic nucleotides deletion	−/+/*
3	N/ predominance fibers I and lipid accumulation in fibers II	ND	p.Tyr430Ser (c.1289A>C) Homozygote	Missense substitution	−/−/*
4	Predominance of type I fibers and atrophy of type II fibers	Abnormally low	c.107–1G>A c.788insC	Splice mutation Frame shift mutation	*/*/*
5	Predominance of type I fibers and atrophy of type II fibers	Abnormally low	p.Tyr430Ser (c.1289A>C) p.Cys427Cys (c.1281C>T)	Missense Substitution. Cryptic splice leading to 19 Nt deletion	*/+/*
6	N	ND	p.Ile446Thr (c.1337T>C) c.1082delC	Missense substitution Frame shift mutation (p.Pro361LeufsX65)	−/*/*
7	N/Predominance of type I fibers and atrophy of type II fibers	ND	c.157dup Homozygote	Frame shift mutation (p.Leu53ProfsX81)	−/−/+
8	ND	ND	p.Pro59Ser (c.175C>T) p.Cys451Ser (c.1351T>A)	Missense substitution	−/*/*
9	ND	ND	p.Arg227X (c.679C>T) Homozygote	Nonsense Mutation	−/−/+
10	ND	ND	p.Trp148X (c.444G > A) Homozygote	Nonsense Mutation	−/−/*
11	Predominance of type I fibers and atrophy of type II fibers	Abnormally low	p.Tyr430Ser (c.1289A>C). Homozygote	Missense substitution	−/*/*
12	Predominance of type I fibers	ND	p.Arg410Trp (c.1228C>T) Homozygote	Missense mutation	*/*/*
13**	Predominance of type I fibers and lipid accumulation in fibers II	ND	c.219+1G>C p.Arg340His (c.1019G>A)	Splice mutation Missense substitution	−/*/*
14**	ND	ND	c.219+1G>C p.Arg340His (c.1019G>A)	Splice mutation Missense substitution	*/*/*
15	Predominance of type I fibers	ND	p.Thr441Ala (c.1321A>G) c.1082del	Missense substitution Frame shift mutation (p.Pro361LeufsX65)	−/+/*

N = "normal; −: absence of clinical efficiency; + = clinical improvement.
* Not administrated; ND: no done.
** Brother and sister.

nerve arborisation in the end plate region. The junctional folds showed marked degenerative changes.

3.2.8. Genetic analysis: Table 1b

In all patients, the molecular defect responsible for the disease was identified by sequencing the 17 exons of *COLQ* gene and their boundaries.

Among the 14 unrelated patients, 16 different mutations were identified. Nine mutations were novel ones including, six null allele c.107−1G>A, c.157dup, c.219+1G>C, c.788insC, c.1082delC, c.1281C>T, and three missense substitutions p.Pro59Ser, p.Thr441Ala and p.Ile446Thr. Seven homozygous mutated patients suggest a familial consanguinity [2,3,7,9–12] including four nonsense mutations and two missense substitutions. The eight remaining patients are heteroallelic for missense and/or null mutations. Interestingly, a novel c.1281C>T mutation was discovered. This substitution is predicted to lead to the synonymous substitution p.Cys427Cys but in fact creates a cryptic splice donor site in exon 16 leading to an aberrant splicing and the deletion of 19 nucleotides in exon 16 confirmed by mRNA analysis (data not shown).

4. Discussion

We have reported data on 15 *COLQ*-mutant CMS followed up, over 10 years on average (Table 2). At the end of our study, 80% of patients were ambulant without limited area and 87% of patients did not need any assistance to breathe. However, relapses lasting up to several years have been reported in our whole cohort. They were characterized by worsening of muscle weakness with or without bulbar signs. Puberty and pregnancy were identified as triggering factors. In addiction, hormonal contraception treatment or occurrence of the menopause stabilized or even improved the clinical status highlighting the implication of hormonal factors in the variability of symptoms in *COL Q*-related CMS. Premenstrual syndrome and menstruations has already been reported as triggering factor of weakness worsening in myasthenia gravis [24]. Microphysiological study of endplate potentials in frog skeletal muscle has suggested that progesterone can naturally modulate neuromuscular transmission through the raising of acetylcholine esterase [25]. Worsening of muscle weakness and increased jitter during menstruations were also observed in a case of anti-MuSK positive female patient [26].

Table 2
Comparison of our results with the literature [12].

Clinical symptoms or EMG findings	Literature (%)	Our study (%)
Proximal muscle weakness	100	100
Lack of benefit from ACHE inhibitors	100	100
Decrement	89	100
Onset at birth or during the first year of life	81	47
Ptosis	81	53
Delayed motor milestones	77	27
Double CMAP	66	100
Facial weakness	63	33
Axial muscle weakness	63	80
Ophthalmoparesis	59	47
Respiratory crises	45	33
Dysphagia and/or chewing difficulties	40	47
Slow pupillary light response	25	20
Pains	nd	87

Diagnosis was delayed in the whole cohort with a mean age of 23 years after the onset of the symptoms which was at 13 months of age on average. The mean age at the first examination was 19 years (3 to 48 years old), which may explain the delay of the diagnosis. EMG carried out in 93% of our cohort, showed a double CMAP in response to single nerve stimulus in all cases. This result allowed us to avoid other congenital myasthenic syndromes with mutations in RAPSN, CHAT and DOK7. Double CMAP is characteristic but not specific of a Col Q related CMS. It has also been observed in Slow-channel CMS [1]. However, there is usually an autosomal dominant heredity except rare cases of recessive Slow-channel CMS where onset of symptoms is later in young adults [27].

We tried to determine if a correlation could be evidenced between the phenotype and the genetic defect i.e., the nature of the mutation (null allele versus missense substitution) or the location of the mutation in the protein domains. In our study, all patients but one (patient 2) presenting two null alleles leading to an absence of protein

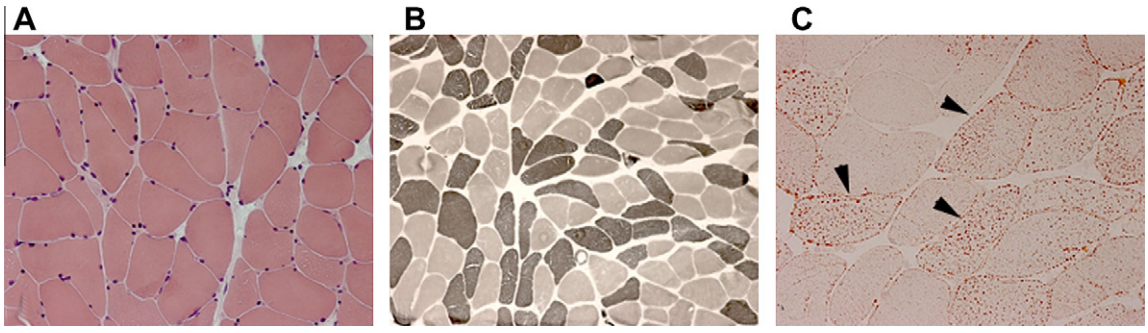

Fig. 2. Muscle biopsy from the deltoid muscle (A–C: ×200). (A) Haematoxylin-eosin stain showing atrophic fibers and variation in the fiber size. (B) ATPase reaction at ph 9.4 showing type I fiber predominance. (C) Oil Red O staining showing increased lipid droplets in muscle fibers type II (arrows).

synthesis were more severely affected than patients with one or two missense alleles, corroborating the data of precedent studies [12]. No correlation could be clearly observed between the location of the missense mutations within the protein and the phenotype. Among *COLQ* mutated patients, only the p.Tyr430Ser substitution (homozygous in patient 11 and compound heterozygous in patient 5) might be associated with later onset, a slow progression and a relatively mild "limb-girdle" phenotype [9,12,21].

Another triggering factor of relapses identified was potentially a treatment by esterase inhibitors corroborating previous studies [8,9,15,19–21] whereas ephedrine had a remarkable benefit on muscle weakness and respiratory function as in other reports [12,21–23]. This treatment showed in a few days an improvement of the clinical status in both cases: relapse or even progressive worsening.

Finally, in order to shorten the diagnostic delay and avoid esterase inhibitors which could dramatically worsen patients, we recommend an EMG with the study of the neuromuscular transmission. Then, diagnosis will be easily confirmed by the molecular genetic analysis. Regarding to the remarkable benefit of ephedrine, we suggest the use of this treatment at puberty or earlier in case of relapse.

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