doi:10.1093/brain/awm325 *Brain* (2008), **131**, 747–759

Clinical and molecular genetic findings in COLQ-mutant congenital myasthenic syndromes

Violeta Mihaylova,[1,†] Juliane S. Müller,[1,*,†] Juan J. Vilchez,[2] Mustafa A. Salih,[3] Mohammad M. Kabiraj,[4] Adele D'Amico,[5] Enrico Bertini,[5] Joachim Wölfle,[6] Felix Schreiner,[6] Gerhard Kurlemann,[7] Vedrana Milic Rasic,[8] Dana Siskova,[9] Jaume Colomer,[10] Agnes Herczegfalvi,[11] Katarina Fabriciova,[12] Bernhard Weschke,[13] Rosana Scola,[14] Friederike Hoellen,[1] Ulrike Schara,[15] Angela Abicht[1] and Hanns Lochmüller[1,*]

[1]Friedrich-Baur-Institute, Department of Neurology, Ludwig-Maximilians-University, Munich, Germany, [2]Servicio de Neurologia, Hospital Universitari La Fe, Valencia, Spain, [3]Division of Pediatric Neurology, College of Medicine, [4]Division of Neurophysiology, Department of Neurosciences, Armed Forces Hospital, Riyadh, Saudi Arabia, [5]Laboratory of Molecular Medicine, Bambino Gesu Children's Hospital, Rome, Italy, [6]Children's Hospital, University of Bonn, [7]Department of Neuropediatrics, Universitäts-Kinderklinik Münster, Germany, [8]Clinic for Child Neurology and Psychiatry, Medical University Belgrade, Serbia, [9]Department of Pediatric Neurology, University Hospital 'Thomayerova', Prague, Czech Republic, [10]Unitat de Patologia Neuromuscular, Servei de Neurologia, Hospital Sant Joan de Déu, Esplugues (Barcelona), Spain, [11]Department of Neurology, Bethesda Children's Hospital, Budapest, Hungary, [12]University Children's Hospital, Bratislava, Slovakia, [13]Department of Neuropediatrics, Medical Faculty of the Charite, Humboldt University, Berlin, Germany, [14]Neuromuscular/Neurology Division, Hospital de Clinicas, Universidade Federal do Parana, Curitiba, Brazil and [15]Department of Pediatric Neurology, University of Essen, Germany

[*]Present address: Institute of Human Genetics, University of Newcastle upon Tyne, Newcastle upon Tyne, UK

[†]These authors contributed equally to this work.

Correspondence to: Hanns Lochmüller, MD, Institute of Human Genetics, University of Newcastle upon Tyne, International Centre for Life, Central Parkway, Newcastle upon Tyne NEI 3BZ, UK
E-mail: hanns.lochmuller@ncl.ac.uk

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 synaptic basal-lamina associated CMS with end-plate AChE deficiency. Here we present the clinical and molecular genetic findings of 22 *COLQ*-mutant CMS patients, carrying a total of 20 different *COLQ* mutations, 11 of them had not previously been reported. Typically, patients with esterase deficiency suffer from a severe, progressive weakness with onset at birth or in early infancy. In addition, patients with a late onset showing a mild course of disease are described. AChE inhibitor therapy, beneficial for other forms of CMS, is of no effect in cases of esterase deficiency. The large cohort of *COLQ* patients studied here enabled us to define additional clinical presentations associated with *COLQ* mutations that differ from the 'classical' phenotypes: several patients with disease onset at birth or in early infancy presented an unexpected, mild disease course without significant progression of weakness. Moreover, many patients had clinical features reminiscent of limb-girdle CMS with mutations in the recently discovered *DOK7* gene, including sparing of eye movements and a predominantly proximal muscle weakness. There was no long-term objective benefit from esterase inhibitors treatment in *COLQ* patients. Surprisingly, a short-term beneficial effect was observed in four patients and a Tensilon test was positive in two. Treatment with ephedrine was efficient in all five cases where it was administered. The variability of phenotypes caused by *COLQ* mutations, the divergence from the previously published classical clinical features and an initial positive response to esterase inhibitors in some patients may obscure AChE deficiency as the molecular cause of the disease and delay the start of appropriate therapy. Moreover, overlap with other CMS subtypes and potentially absence of a repetitive compound muscle action potential should be considered in the diagnosis of *COLQ*-mutated patients.

Keywords: congenital myasthenic syndromes; *COLQ*; acetylcholinesterase; esterase inhibitors

Abbreviations: AChE = acetylcholinesterase; CMAP = compound muscle action potential; CMS = congenital myasthenic syndromes; LGM = limb-girdle myasthenia; PRAD = proline-rich region attachment domain; RNS = repetitive nerve stimulation; RSV = respiratory syncytial virus; SC-CMS = slow-channel CMS.

Received July 19, 2007. Revised December 3, 2007. Accepted December 12, 2007. Advance Access publication January 7, 2008

Introduction

Congenital myasthenic syndromes (CMS) are clinically and genetically heterogeneous inherited disorders in which the safety margin of neuromuscular transmission is compromised (overview in: Engel and Sine, 2005; Müller *et al.*, 2007*b*)]. So far, mutations in 10 genes have been identified to cause CMS (*CHAT, COLQ, CHRNA1, CHRNB1, CHRND, CHRNE, RAPSN, MUSK, DOK7, SCN4A*) (Engel and Sine, 2005; Beeson *et al.*, 2006). The syndromes are classified according to the localization of the corresponding defect at the neuromuscular junction as pre-synaptic, synaptic basal-lamina associated and post-synaptic (Engel and Sine, 2005; Müller *et al.*, 2007*b*). Patients usually present with muscle weakness and abnormal fatigability, bulbar and ocular symptoms and respiratory difficulties. The disease course can be stable or progressive leading to considerable disability. The various underlying pathophysiological mechanisms reflecting the diverse genetic defects in CMS lead to different treatment strategies.

The synaptic basal-lamina associated CMS is caused by absence of the asymmetric form of acetylcholinesterase (AChE) from the synaptic space (Donger *et al.*, 1998; Ohno *et al.*, 1998). The absence of the AChE prolongs the lifetime of ACh in the synaptic space, this increases the duration of the end-plate current so that it outlasts the refractory period of the muscle fibre and excites a second compound muscle action potential (CMAP). The prolonged end-plate currents lead to overload of the synaptic space with cations and end-plate myopathy with loss of acetylcholine receptors. Electron microscopy studies of end-plates of patients with *COLQ* mutations revealed degeneration of the junctional folds and abnormally small nerve terminals encased by Schwann cells (Engel *et al.*, 2003*a*).

The asymmetric AChE at the neuromuscular junction is composed of one, two or three homotetramers of globular subunits ($AChE_T$) attached to a triple-stranded collagenic tail (ColQ) (Krejci *et al.*, 1997). Each ColQ molecule can bind one $AChE_T$ tetramer. The ColQ protein is composed of an N-terminal proline-rich region attachment domain (PRAD), a collagenic central domain and a C-terminal region enriched in charged residues and cysteines. Two heparan sulphate proteoglycan binding domains in the collagen domain and the C-terminal domain anchor the enzyme in the synaptic space (Deprez *et al.*, 2003; Kimbell *et al.*, 2004; Guerra *et al.*, 2005).

End-plate AChE deficiency is not caused by mutations in the *ACHE* gene encoding the catalytic subunit, but by recessive mutations in the *COLQ* gene encoding the collagenic tail subunit. No fewer than 30 different *COLQ* mutations have been identified to date; the majority of them are frameshift or nonsense mutations and truncate the protein distally to PRAD (Ohno *et al.*, 2000; Engel *et al.*, 2003*b*; Engel and Sine, 2005).

Most described patients with mutations in *COLQ* are severely disabled from an early age with respiratory difficulties and progressive involvement of the axial muscles leading to severe scoliosis and restrictive ventilatory deficit. Repetitive CMAP, slow pupillary light response, no effect or even worsening after administration of AChE inhibitors are considered as clinical clues pointing to the diagnosis (Engel *et al.*, 2003*b*).

Here, we present the clinical and molecular genetic findings of 22 patients with *COLQ* mutations. The considerable size of the group studied enables us to further define the phenotypic and genotypic spectrum of end-plate AChE deficiency. We observed a large clinical heterogeneity and in a considerable number of patients absence of classical clinical symptoms, that together may give rise to diagnostic difficulties neurologists and paediatricians are likely to face.

Patients and Methods

The study included 22 patients (males nine, females 13) from 20 unrelated families. Patients 7 and 8 (deceased), as well as Patients 15 and 21 are siblings. Nine patients (1, 10, 12, 15, 17, 18, 20, 21, 22) were born from consanguineous marriages. Patients 7 and 8 were previously published (Schreiner *et al.*, 2007). Detailed description of the clinical features, electrophysiological and morphological end-plate studies of Patient 12 can be found elsewhere (Weschke *et al.*, 2007, submitted). Written informed consent was obtained from all patients, siblings and parents. The study complies with the ethical guidelines of the institutions involved.

Detailed neurological examination and electrophysiological studies including 3 Hz repetitive stimulation of proximal (deltoid or trapezius) and distal (abductor pollicis brevis and/or abductor digiti minimi) muscles were performed. Muscle biopsy specimens were obtained from 11 patients (2, 3, 7, 9, 10, 11, 16, 18, 19, 20, 22).

Venous blood samples were drawn for DNA extraction from the patients and their unaffected relatives. Genomic DNA was isolated using blood DNA extraction kit according to the manufacturer's recommendations (Promega, Mannheim, Germany). DNA from Patient 8 was extracted from fixed liver tissue.

In all patients the 17 *COLQ* exons and the flanking intron regions were amplified by PCR and sequenced (primers available

upon request). The Genbank reference numbers used for comparison of the genomic sequence of the *COLQ* exons are AF229117-AF229126; the reference number for the mRNA sequence is gi3378117. PCR-amplified fragments were purified with the NucleoSpin Extract Kit (Macherey-Nagel, Düren, Germany) and sequenced using an Applied Biosystems model 3700 or 3730XL DNA Analyzer and fluorescence-labelled dideoxy terminators (BigDye Terminator v3.1 Cycle Sequencing Kit, Applied Biosystems). For each of the newly identified mutations, screening among at least 60 control individuals by direct sequencing was executed.

Results

Clinical phenotype

Age of onset and clinical symptoms

In the majority of the patients, the disease manifests at birth (11 patients). Muscle hypotonia, ptosis, ophthalmoparesis, poor cry and suck, respiratory insufficiency were the reported presenting symptoms in the neonatal period. The disease onset was within the first year of life in seven patients and the most common presenting sign was poor head control. In four patients, the initial symptoms were noted after the first year of life. Muscle weakness and/or fatigability were the initial symptoms for Patients 6, 11 and 14 at age 6, 7 and 2 years, respectively. Patient 4 presented with fatigability and frequent falls at age 6 years. When tired, Patients 4 and 11 developed postural scoliosis and crossed their legs probably for balancing the trunk, similar to a previously described patient [Patient 2 in (Hutchinson *et al.*, 1993)].

Delayed motor developmental milestones were frequently observed (17/22 patients). The three patients with disease onset during preschool age had achieved normal motor skills. Despite the disease onset during the first year of life, two patients were able to walk normally at age 12 and 14 months.

Respiratory crises were common among our patients: 10 patients experienced respiratory crises, which were precipitated by infections in five of them. Four patients (1, 2, 3, 16) presented with respiratory distress at birth and were temporarily ventilated. Respiratory difficulties were serious in seven patients and lethal in two of them. Patient 8 developed respiratory insufficiency in the course of a minor infection at age 3 years and was intubated. Subsequently he developed pseudomonas sepsis. Intermittent spontaneous breathing occurred after 4 weeks of assisted ventilation. Two months later, the patient rapidly deteriorated and died. Patient 2 (Fig. 1C), at age 3.5 years, died during respiratory crisis. The post-mortem examination disclosed pulmonary hypertension and enlarged right ventricles.

After a severe episode of respiratory distress, a 1-year-old male patient was tracheostomized (Patient 3, Fig. 1I–K). Now, at age 27 months, he needs nocturnal ventilatory support. The recurrent episodes with respiratory insufficiency in Patient 16 also led to the placement of tracheostoma (Fig. 1E). Now, at age 15 years, the patient requires nocturnal ventilation. Two further patients (4 and 20) are also ventilatory dependent since the age of 16 and 12 years, respectively, but currently are ventilated only during the night. Patient 17, aged 14 years, has needed nocturnal ventilatory support for 7 months.

Clinical signs of hypoventilation were noted in three patients (1, 12, 22) without mechanical ventilation being started, yet. Patients 1 and 12 had a history of recurrent chest infections. Patient 22 (Fig. 1F and G) reported dyspnoea on exertion. Polysomnography was performed in Patients 1 and 20 and revealed frequent episodes of sleep apnoea.

Generalized muscle weakness was found in 19 patients. In three patients, the weakness was predominantly or exclusively limited to proximal and axial muscles (Patients 6, 10, 11) (Figs 1A, B, D and 2A, C–E). The weakness was moderate in 8 out of 20 patients (1, 2, 3, 7, 8, 13, 18, 22), mild in 8 out of 20 (patients 5, 6, 10, 11, 12, 14, 15, 19) and severe in four out of 20 (patients 4, 16, 17, 20), rendering two patients wheelchair bound (4 and 16) at age 15 years and 9 years, respectively and two others with restricted ambulation (17 and 20).

Facial weakness was observed in 14 patients (Fig. 1C, E and F). Bilateral ptosis and ophthalmoparesis were found in 13 patients (Figs 1C, F, I and 2F). Ophthalmoparesis was complete in three patients (5, 18, 22), the ptosis was asymmetric in Patients 1, 11 and 18 (Fig. 2E and F). Eye-lid drooping with spared extra-ocular movements was found in five patients (Fig. 2C–E). Slow pupillary response to light was not a consistent finding, as it was found in 5 out of 20 patients, only (Fig. 2B). Dysphagia was found in seven patients. Chewing difficulties were reported in three patients (8, 18, 20).

Proximal muscle weakness was encountered in all patients. Seven patients had scapular winging (Fig. 1G). The gait was waddling in nine patients. Interestingly, two patients (6, 12) had inward rotation of knees in addition to the waddling gait (Fig. 1D). Distal muscle weakness was observed in seven patients and was equal to proximal weakness in four of them. Patient 18 showed predominant involvement of upper limb muscles with pronounced weakness of finger and wrist extensors. Selective involvement of neck muscles (flexors or extensors) was noted in 10 patients (Fig. 1C). Muscle atrophies were found in six patients. The tendon reflexes were absent in Patients 5 and 16 and diminished in Patients 1, 2, 4, 7, 8, 17, 20, 22. Axial muscle weakness with or without scoliosis (kyphosis) was found in 14 patients (Fig. 1G).

Arthrogryposis was not recorded in any of the patients. Dysmorphic features were rarely observed: high-arched palate was noted in three patients and one of them had also triangular mouth and prognathism. Additional findings were congenital clubfeet (Patient 3) and mental retardation (Patients 16 and 22) (Fig. 1J).

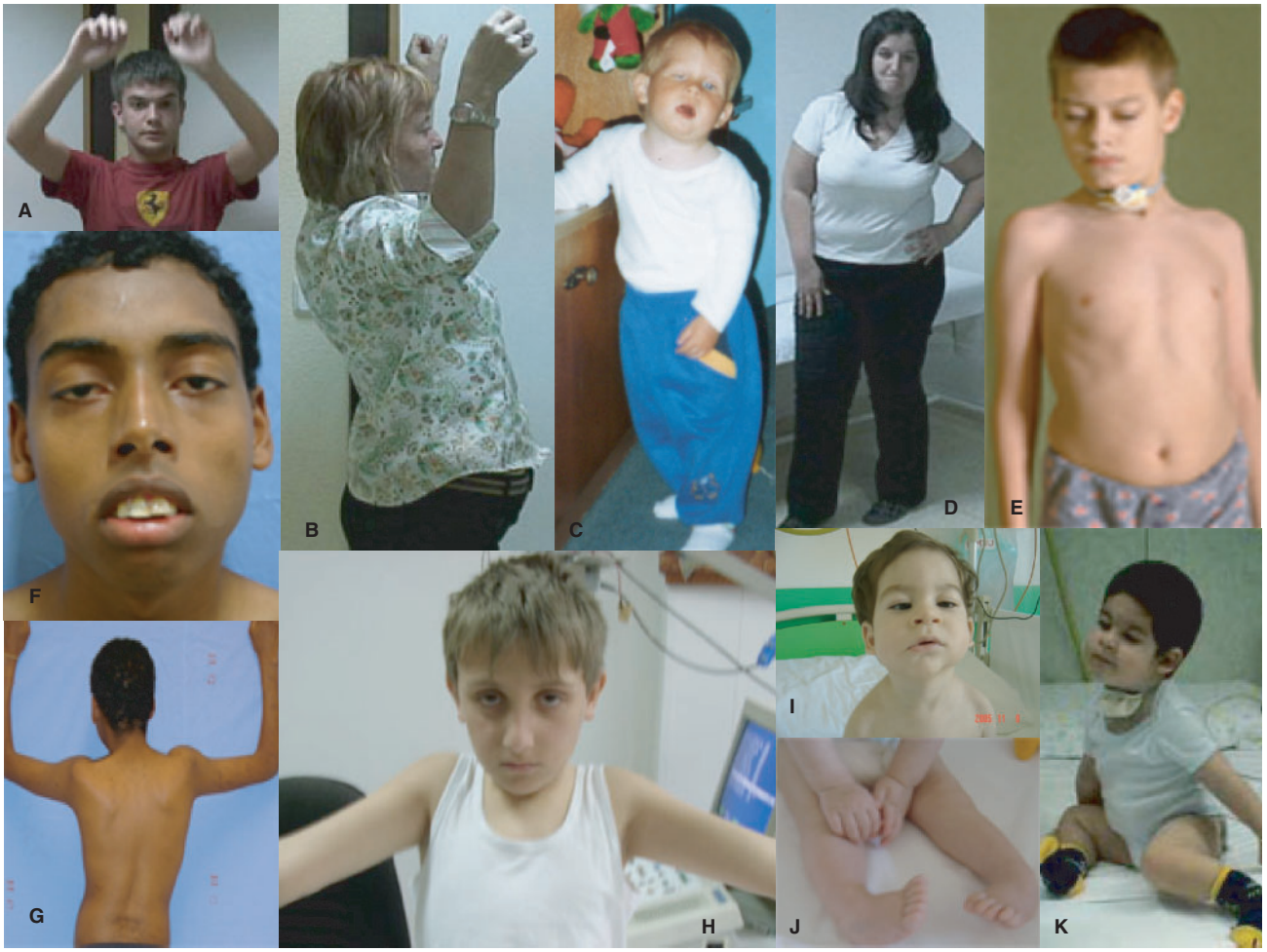

Fig. 1 Patients with *COLQ* mutations. (**A**) Patient 10. He is only mildly affected. (**B**). Patient 11. Note the hyperlordosis. (**C**) Patient 2. She has neck muscle weakness, facial weakness, ptosis and ophthalmoparesis. (**D**) Patient 6. Note the inward rotation of the knees. (**E**) Patient 16. He has tracheostoma, facial weakness, ptosis, ophthalmoparesis, severe muscle weakness. (**F** and **G**) Patient 22. He has ptosis, ophthalmoplegia, facial weakness, scapular winging and scoliosis. (**H**) Patient 14. He has ptosis and proximal muscle weakness. (**I** and **J**) Patient 3 at 1 year of age. Note the congenital clubfeet, ptosis and ophthalmoparesis. (**K**) Patient 3 at 2 years of age. He has a tracheostoma.

Diurnal fluctuation of symptoms was evident in eight patients. Interestingly, two patients (Patients 6 and 11) had fluctuation of the symptoms over longer periods (weeks and months). Though Patient 6 is able to walk hundreds of meters, there are weeks when she needs support or even wheelchair for the same distance. Patient 11 reported 'bad days' when she was not able to take part in her favourite activities.

Progression of the disease was noted in nearly half of the Patients (9/21 patients). While the disease course was slowly progressive over a period of more than 5 years in some of the patients (4, 5, 9, 16, 17, 20, 22), the progression was relatively rapid over a period of 3 years in Patient 2 and several months in Patient 8.

The individual clinical data are summarized in Table 1.

Electrophysiological studies

Decremental response following repetitive nerve stimulation (RNS) was found in 17 out of 19 patients. No decrement was recorded from distal and/or proximal muscles in two patients (15, 17) aged 2 and 6 years. Double CMAP in response to single nerve stimulus was evident in 12 out of 18 patients and became triple following administration of 3,4-DAP and Tensilon in Patient 6 (Fig. 3). Single CMAP was recorded in six patients aged 6 months, 1, 3, 6, 12, 22 years. However, double CMAP after administration of 3,4-DAP and Tensilon was recorded in one of them (Patient 4 at 22 years of age). SF-EMG disclosed increased jitter in all three patients in whom it was performed. Needle EMG was executed in 15 patients and revealed myopathic potentials in nine.

Laboratory studies and muscle biopsy specimens

AChR antibodies were not detected in any of the patients in whom testing was performed (8/8). The CK level was normal in all individuals tested (14/14). Muscle biopsy

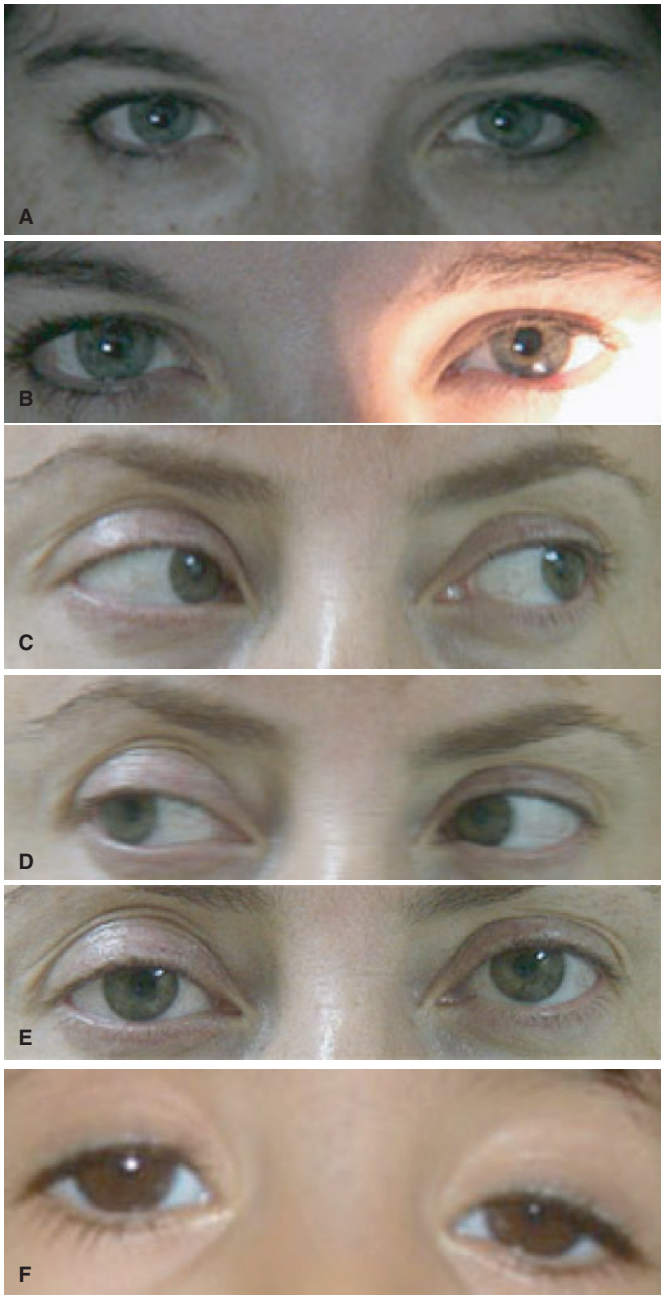

Fig. 2 Ocular muscles in *COLQ*-mutant patients. (**A**) Patient 6. Spared ocular muscles; (**B**) Patient 6. Delayed pupillary light response; (**C**–**E**). Patient II. She has asymmetric ptosis, but no ophthalmoparesis. (**F**) Patient I. She has asymmetric bilateral ptosis and ophthalmoparesis.

specimens were examined in 11 patients and were unremarkable in four (Patients 3, 11, 16 and 22 at age 1 year, 13 years, 4 months and 7 years, respectively). Unspecific myopathic changes were noted in four patients (Patients 7, 9, 10 and 20) together with fibre type I predominance in Patients 7 and 10. ATPase pH 4.3 stain demonstrated type IIC fibres in Patient 10. Fibre type I predominance without any other abnormalities was repeatedly found in Patient 18 at age 1 and 11 years and in Patient 2 during infancy. However, post-mortem examination of the muscles demonstrated myopathic changes in the latter. Mild lipid accumulation together with mild type II fibre atrophy were detected in Patient 19 at age 2 years.

Response to AChE inhibitors

Generally, there was either no long-term beneficial effect from AChE inhibitor treatment or worsening of the symptoms. However, short-term positive response to AChE inhibitors was reported in four patients, followed by deterioration and discontinuation of the treatment. Patient 22 reported sustained benefit from pyridostigmine treatment (240 mg/day) over a period of 10 years, but no objective clinical improvement was observed. Clear worsening of the symptoms after administration of AChE inhibitors was seen in nine out of 19 patients; no clear effect on clinical signs was evident in five cases. In Patient 12, a reduction of the decrement from 39 to 10% following oral administration of 20 mg pyridostigmine was noted. Tensilon test was performed in four patients: the result was positive in two (Patients 12 and 15), no change or muscarinic adverse effects were observed in Patients 7 and 2, respectively.

Ephedrine was given to five patients. An improvement of the clinical symptoms following administration of ephedrine (2.2 mg/kg/day) was reported for Patient 1, while recovering from RSV (Respiratory Syncytial Virus) infection without requiring assisted ventilation. In Patient 3, treatment with ephedrine (3 mg/kg/day) was started and surgery for the clubfeet deformity was performed at age 2 years: 3 months later the child was able to walk with support and required only nocturnal ventilatory support, while ptosis and ophthalmoparesis remained unchanged. In Patient 4, ephedrine treatment started at the beginning of the year and resulted in increased FVC from 40 to 60%. Now the patient is on 150-mg/day ephedrine (combined with beta-blocker) and is able to walk 200 m independently and no respiratory crises have been observed for 6 months. In Patient 17, 6 weeks of ephedrine treatment (2 mg/kg/day) led to remarkable increase in walking distance. In Patient 20, 200 mg/day of ephedrine improved his general well-being and abolished the need for oxygen during the day. 3,4-DAP was given to six patients (4, 6, 9, 10, 11, 12) with no beneficial effect in all but one. Patient 6 has been treated since the age of 17 years with 30–60 mg 3,4-DAP daily (up to the patient's discretion) followed by objective clinical improvement: increased exercise tolerability and longer walking distance.

Genetic analysis

Twenty different mutations of the *COLQ* gene have been identified in our patients, 11 of them have not been reported so far. The mutations are missense (6), splice-site (3), in-frame deletion (1), non-sense (4) and frameshift (6). The majority of the mutations are protein truncating and are located in the C-terminal and collagen domain of ColQ. Only four mutations are located in PRAD (Fig. 4, Table 1).

Table 1 Individual clinical and genetic data of the 22 patients with *COLQ* mutations

Patient	Sex	Ethnic origin	Age of onset (years)	Delayed motor milestones	Respiratory crises	Ptosis/ophthalmoparesis/facial weakness/dysphagia	Slow pupillary light response	Proximal weakness/waddling gait/scapular winging/atrophies	Distal muscle weakness/selective involvement of wrist and finger extensors	Axial/neck muscles weakness/ scoliosis or kyphosis	Abnormal tendon reflexes	Progressive disease course	Disease severity	Electrophysiological studies: RNS decrement	Electrophysiological studies: Double CMAP	Electrophysiological studies: Myopathic potentials	Negative or ambiguous response to acetylcholinesterase inhibitors	Mutations
1	F	Saudi Arabian	Birth	+	+	+/+/+/+	–	+/+/+/–	–/–	+/+/+	+	–	Mod	+	+	ND	+	**C417Y/C417Y**
2[a]	F	Hungarian	Birth	+	+	+/+/+/+	–	+/–/+/+	–/–	–/+/–	+	+	Sev	ND	ND	+	+	Q211X/**IVSI5 + IG>T**
3	M	Italian	Birth	+	+	+/+/–/+[b]	–	+/–/–/–	–/–	+/–/–	–	[c]	Sev	+	–	ND	+	I082delC/I082delC
4	F	Spanish	6	–	+	+/–/+/+	–	+/+/–/–	–/–	–/–/–	+	+	Sev	+	–	–	+	Y430S/Y430S
5	F	Turkish	<1	+	–	+/+/+/–	–	+/–/–/–	–/–	–/+/–	+	+	Mild	+	–	ND	±	I082delC/I082delC
6	F	Spanish	6	–	–	–/–/–/–	–	+/+/+/–	–/–	–/–/–	–	–	Mild	+	+	–	+	Y430S/Y430S
7	F	German	Birth	+	+	+/+/+/–	–	+/–/–/–	+/–	–/–/–	+	–	Mod	+	+	ND	+	IVSI-IG>A/950delC
8[a]	M	German	Birth	+	+	+/+/+/–	–	+/–/–/–	+/–	–/–/–	+	+	Sev	ND	ND	ND	NT	IVSI-IG>A/950delC
9	F	German	<1	+	–	–/–/–/–	nd	+/–/–/–	+/–	–/+/–	–	+	Mild	+	+	–	+	T441A/T441A
10	M	Spanish	<1	–	–	–/–/–/–	–	+/+/–/+	–/–	+/–/–	–	–	Mild	+	+	+	+	Y430S/Y430S
11	F	Spanish	7	–	–	+/–/–/–	–	+/–/–/–	–/–	+/–/–	–	–	Mild	+	+	+	+	Y430S/Y430S
12	F	Lebanese	<1	–	–	–/–/–/–	–	+/+/–/–	+/–	+/+/+	–	–	Mild	+	+	+	[d]	R341G/R341G
13	M	Spanish	Birth	+	–	+/–/–/+	+	+/–/–/–	–/–	+/+/–	–	–	Mod	+	–	+	+	**I58insC/I58insC**
14	M	Serbian	2	+	–	+/–/–/–	–	+/–/–/–	–/–	+/–/+	–	–	Mild	+	+	+	+	**I09delC/I324delCAG**
15	F	Pakistanian	<1	+	–	+/–/+/–	nd	+/–/–/–	–/–	+/+/+	–	–	Mild	–	ND	+	±	**R227X/R227X**
16	M	German	Birth	+	+	+/+/+/–	+	+/+/+/–	–/–	+/+/+	+	+	Sev	+	+	–	±	W148X/**C386S**
17	F	Turkish	Birth	+	+	+/+/+/–	–	+/–/–/–	+/–	+/–/+	+	+	Sev	–	–	–	±	R236X/R236X
18	F	Italian	Birth	+	–	+/+/+/–	+	+/+/+/+	+/+	+/+/–	–	–	Mod	+	+	ND	+	I082delC/I082delC
19	M	Czech	Birth	+	–	+/+/+/–	–	+/–/+/+	–/–	+/–/–	–	–	Mild	+	–	–	+	**G237D/797insC**
20	M	Saudi Arabian	Birth	+	+	+/+/+/+	–	+/+/–/+	–/–	+/–/+	+	+	Sev	+	+	+	NT	**738delA/738delA**
21	F	Pakistanian	<1	+	+	+/+/+/+	+	+/na/–/–	–/–	–/+/–	–	–	Mod	ND	ND	ND	NT	**R227X/R227X**
22	M	Brazilian	<1	+	–	+/+/+/–	+	+/+/+/+	+/–	+/–/+	+	+	Mod	+	+	+	–	**IVS2+IG>C/IVS2+IG>C**

+ = yes; – = no; ± = denotes initial positive response followed by ineffectiveness and worsening; ND = no data; NT = not tried; NA = not applicable; mod = moderate; sev = severe. [a]Deceased. [b]During episodic crises. [c]Placed on ephedrine treatment in early childhood. [d]Reduction of the decrement, but no effect on clinical signs. The newly identified mutations are in bold and underlined.

All patients carried either homozygous or compound heterozygous mutations. Molecular genetic testing of the family members was compatible with a recessive trait for all mutations, i.e. the parents of each patient carry single mutant allele and the unaffected siblings harbour either one or no mutant allele. Sixteen patients from 15 unrelated families are homozygous for *COLQ* mutations; nine of them belong to consanguineous families.

We describe three new missense, one new in-frame deletion and seven new truncating mutations.

The newly identified missense mutations *C417Y, C386S, G237D* were not detected in 120 normal alleles. The mutations *C417Y* and *C386S* lead to the substitution of conserved cysteine in the C-terminal domain for tyrosine and serine, respectively. The charged residues and cysteines of the C-terminal domain could participate in anchoring ColQ to the basal lamina by electrostatic interactions or by disulphide bonding (Ohno *et al.*, 2000). Four previously reported insertion incompetent mutations eliminate either cystein or a charged residue in the C-terminal domain (Ohno *et al.*, 2000; Kimbell *et al.*, 2004). The *G237D* mutation leads to the substitution of a highly conserved residue in one of the heparin sulphate proteoglycan-binding domains which are also involved in attachment of the ColQ to the basal lamina.

The splice-site mutation *IVS15 + 1G > T* is likely to affect splicing. Exchange of the nucleotide G at the same position for the nucleotide A was previously reported in CMS (Ohno *et al.*, 2000). The end-plates of the patients harbouring IVS15 + 1G>A had only trace amounts of AChE. In addition to the insertion incompetence, the sedimentation profiles obtained from COS cells transfected with wt $AChE_T$ and cDNA lacking exon 15 revealed a distinct 10.5S mutant peak pointing to impaired triple helix formation (Ohno *et al.*, 2000).

The splice-site mutation *IVS2 + 1G > C* is likely to be pathogenic. It was not found in 120 normal alleles. The parents of the patient are related and both carry the mutation heterozygously. None of the six healthy siblings carries the mutation homozygously.

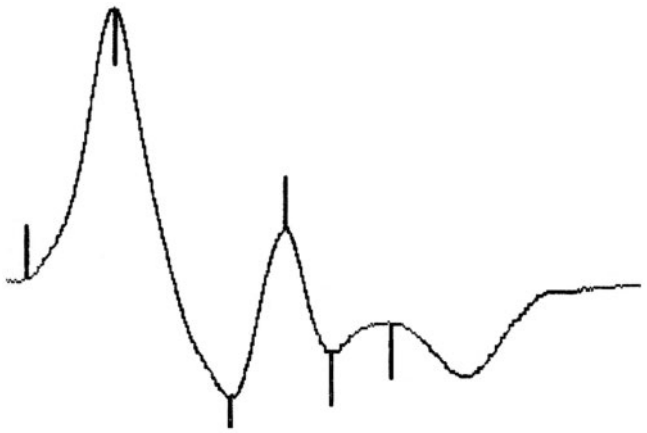

Fig. 3 Triple CMAP following administration of 3,4-DAP and Tensilon in Patient 6.

Discussion

Here, we present the clinical and molecular genetic findings of 22 *COLQ*-mutant CMS patients representing 5% of our CMS patient cohort and about 10% of our CMS patients with established genetic diagnosis. To our knowledge, this is the first time such a large number of CMS patients with *COLQ* defects were compiled in a single study. Furthermore, many of these patients were followed over a period of several years allowing assessment of disease course and progression. The large number of patients enabled us to define clinical phenotypes not linked to AChE deficiency previously, as well as conduct genotype–phenotype correlation and compare the clinical phenotype of *COLQ* patients to the phenotypes of CMS patients with mutations in other genes.

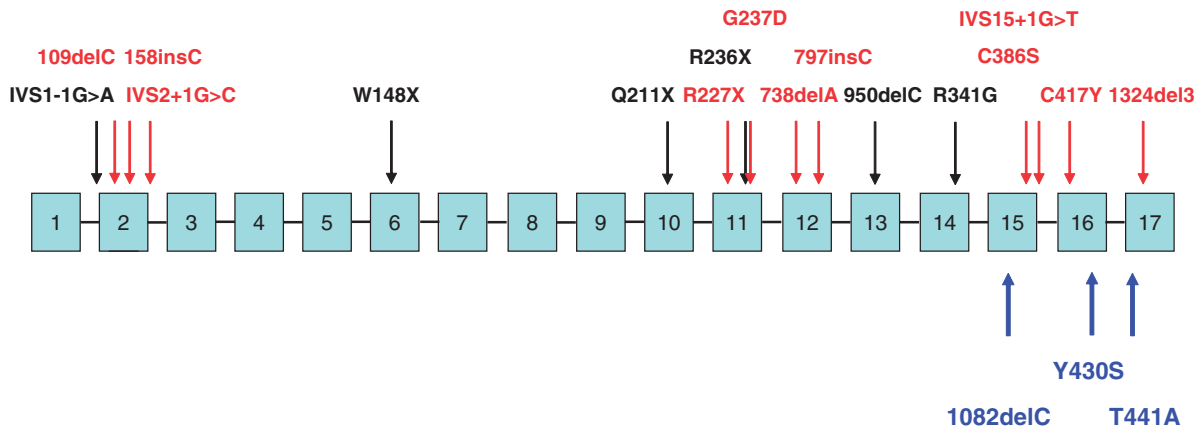

Fig. 4 Mutations in *COLQ* gene identified in our patients. The blue boxes represent the exons, but do not reflect the exons' length. The location of the mutations is indicated by arrows. The novel mutations are marked in red; the recurrent mutations in *COLQ* in our CMS patients cohort are marked in blue.

Comparison of *COLQ* phenotypes

Typically, the disease onset was at birth or during infancy and the latest reported age of onset was 7 years. The early motor development was delayed in the majority of the patients (Table 3). Respiratory difficulties were also frequently observed in our cohort, as they were evident in half of the patients and were severe enough to cause death or need of assisted ventilation. Similarly to other neuromuscular disorders, episodes of sleep apnoea can be observed in CMS patients, as it was documented in two of our patients. Given the serious consequences of sleep apnoea (e.g. cor pulmonale), polysomnography may be performed in all *COLQ* patients.

Facial weakness was observed in more than half of the patients. Swallowing difficulties were not frequent as they were reported in less than half of the patients. Involvement of ocular muscles was common: ptosis was found in 18 patients and extra-ocular movements were limited in more than half of the patients (Table 3). Although the asymmetric ptosis is more frequently associated with autoimmune myasthenia gravis, it was found in three of our *COLQ*-mutant patients and previously reported in two cases [Table 2, (Hutchinson *et al.*, 1993; Beeson *et al.*, 2005)]. Delayed pupillary light response is one of the clinical clues for the diagnosis (Engel *et al.*, 2003*a*). However, it was only observed in 25% of our patients (Table 3).

The muscle weakness was usually generalized, though some patients demonstrated weakness restricted to selected muscle groups. The proximal muscles were more frequently affected than the distal ones. The waddling gait, muscle atrophies, scapular winging and diminished reflexes observed in some of the patients give rise to the impression of myopathy rather than of CMS.

Evidence of impaired neuromuscular transmission was demonstrated by decremental response on RNS and by increased jitter in SFEMG in 17 out of 19 patients. The characteristic double CMAP was observed in more than half of the patients (Table 3), but was not recorded in six patients, which cannot be exclusively attributed to the early age of the examination, as it was not elicited even in a 22-year-old patient (Patient 4). However, it was observed after administration of 3,4-DAP and Tensilon in the latter. These agents also elicited triple CMAP in Patient 6, highlighting the value of CMAP recording after Tensilon test.

Lack of long-term benefit from esterase inhibitors treatment was the most consistent finding in our patients (Table 3). However, initial positive response was observed in 21% of the patients and one patient even reported long-term benefit. Though esterase inhibitors should have no effect on the decrement in end-plate AChE deficiency, reduction of the decrement following administration of pyridostigmine was observed in one of our patients and was previously reported in another (Bestue-Cardiel *et al.*, 2005). Beneficial effect from ephedrine treatment was observed in five patients, in whom it was administered, reducing the oxygen dependence and making ICU admissions due to respiratory insufficiency unnecessary. Benefit from ephedrine treatment was previously observed in two other *COLQ*-mutant patients (Bestue-Cardiel *et al.*, 2005). The increased walking distance together with improved ventilatory function in *COLQ*-mutant patients following ephedrine treatment are important observations both for clinicians and for the patients as this is a frequently disabling disorder and no other treatment options are available.

Two clinical phenotypes of patients with *COLQ* mutations had been published, previously: the 'classical' *COLQ* phenotype with the hallmarks disease onset at birth, moderate to very severe weakness and no response to esterase inhibitor therapy (Table 2) (Ohno *et al.*, 1998, 2000; Shapira *et al.*, 2002). A different phenotype has been associated to the missense mutations Y430S (Donger *et al.*, 1998; Bestue-Cardiel *et al.*, 2005), and T441A (Müller *et al.*, 2004) and one patient carrying G240X (Shapira *et al.*, 2002): disease onset at 6–7 years of age, mild weakness and slow or no progression of symptoms and no response to AChE inhibitors (Table 2). Cranial muscles are usually spared in this patient group. Some of our *COLQ* patients correspond to one of the two phenotypes e.g. Patients 6, 11 and 14 to the milder phenotype with onset after the first year of life, and e.g. Patients 1, 2, 3, 7, 8, 13, 18, 20 and 21 to the early onset severe phenotype. In addition, we observed two additional phenotypes: Patients 5, 9, 10, 12, 15 and 19 had a disease onset at birth or in early infancy, but the disease course was non-progressive and the weakness remained mild. Patient 4, on the other hand, had a late disease onset but suffers from a very severe and progressive course.

All previously reported AChE deficient patients have either negative Tensilon test or failed to respond to esterase inhibitors treatment (Hutchinson *et al.*, 1993; Donger *et al.*, 1998; Ohno *et al.*, 1999; Ishigaki *et al.*, 2003; Müller *et al.*, 2004; Bestue-Cardiel *et al.*, 2005). Intriguingly, five of our patients (5, 12, 15, 16, 17), showed an initially positive response to esterase inhibitors treatment or a positive Tensilon test and Patient 22 feels subjective improvement of symptoms during esterase inhibitor therapy. This finding is surprising, as it is thought that esterase inhibitors cannot improve neuromuscular transmission in patients with *COLQ* mutations as they might block residual AChE activity at the patients' end-plates and in addition they inhibit butyrylcholinesterase that partially compensates for absence of AChE. However, it might be possible that—in order to compensate for the lack of esterase—patients' end-plates have adapted by reducing neurotransmitter release or AChR numbers. Given such a situation, administration of esterase inhibitors might lead to an initial improvement of muscle strength.

Table 2 Clinical features of previously published *COLQ*-mutant patients

Published patient	Sex	Ethnic origin	Age of onset (years)	Delayed motor milestones	Respiratory crises	Ptosis/ophthalmoparesis/facial weakness/dysphagia	Slow pupillary light response	Proximal weakness/waddling gait/scapular winging/atrophies	Distal muscle weakness/selective involvement of wrist and finger extensors	Axial muscles weakness/ neck weakness/scoliosis	Reduced tendon reflexes	Progressive disease course	Wheelchair dependence	Electrophysiological studies			Negative response to (or lack of benefit from) acetylcholinesterase inhibitors	Mutations
														RNS decrement	Double CMAP	Myopathic potentials		
1	M	ND	Birth	ND	+	ND/+/ND/ND	+	+/ND/ND/ND	ND/ND	+/ND/+	+	+	+	+	+	ND	+	ND
2	F	ND	Birth	+	+	−/−/+/ND	+	+/ND/ND/+	ND/ND	+/+/+	−	+	−	+	+	ND	+	I082delC/D342E
3	F	ND	Birth	−	−	−/−/+/ND	+	+/ND/ND/ND	+/ND	+/+/+	−	+	−	+	+	ND	+	ND
4	M	ND	Birth	+	+	+/+/ND/+	+	+/NA/−/−	−/−	−/+/−	+	+	NA	+	−	ND	+	R282X/I082delC
5	M	ND	<1	−	−	+/+/+/+	+	+/−/−/−	+/+	+/+/+	−	+	−	+	+	ND	+	ND
6	F	Italian	Birth	ND	+	+/ND/ND/+	+	+/ND/+/+	+/−	+/−/+	−	+	−	+	+	ND	Tensilon test worsening	275insC/Q211X
7	M	Palestinian Arab	Birth	ND	+	+/−/+/+	−	+/−/−/−	+/−	+/+/+	−	+	−	+	+	ND	+	G240X/G240X
8	M	Palestinian Arab	<1	ND	−	−/−/−/−	−	+/−/−/−	+/−	+/−/+	−	+	−	+	+	ND	+ Tensilon test worsening	G240X/G240X
9	F	Palestinian Arab	<1	ND	−	ND/+/−/−	−	+/−/−/−	+/−	+/−/+	−	+	−	+	+	ND	+	G240X/G240X
10	M	Palestinian Arab	<1	ND	−	+/−/−/−	−	+/−/−/−	−/−	−/−/−	−	+	−	+	+	ND	+	G240X/G240X
11	M	Palestinian Arab	<1	ND	+	+/−/−/−	−	+/−/−/−	+/−	ND	−	+	−	+	+	ND	+	G240X/G240X
12	M	Palestinian Arab	2	ND	−	+/−/−/+	−	+/−/−/−	ND	−/−/−	−	+	−	+	+	ND	ND	G240X/G240X
13	F	Iraqi Jew	Early childhood	ND	−	+/+/−/−	NA	+/−/−/−	−/−	+/−/+	−	+	−	−	+	ND	+	G240X/G240X
14–19	(4) M (2) F	Spanish	Childhood	−	−	+(1)/−/−/−	−	−/−/−/−	−/−	−/+(1)/−	−	−	−	+(4)	+ (4)	ND	+	Y430S/Y430S
20	F	ND	<1	−	+	+/+/−/−	+	+/−/−/−	+/−	+/−/−	−	+	Needs a cane	+	+	ND	+	IVS1-1G>A/R236X
21	F	ND	Birth	ND	−	+/+/+/−	+	+/−/−/+	+/−	+/−/−	−	−	−	+	+	ND	+	IVS1-1G>A/788insC
22	M	ND	Childhood	ND	−	−/−/+/−	ND	+/−/−/− Respiratory muscle weakness	−/−	−/+/−	−	+	−	+	+	ND	+	R315X/IVS16+3A>G

(continued)

Table 2 Continued

Published patient	Sex	Ethnic origin	Age of onset (years)	Delayed motor milestones	Respiratory crises	Ptosis/ophthalmoparesis/facial weakness/dysphagia	Slow pupillary light response	Proximal weakness/waddling gait/scapular winging/atrophies	Distal muscle weakness/selective involvement of wrist and finger extensors	Axial muscles weakness/ neck weakness/scoliosis	Reduced tendon reflexes	Progressive disease course	Wheelchair dependence	Electrophysiological studies: RNS decrement	Electrophysiological studies: Double CMAP	Electrophysiological studies: Myopathic potentials	Negative response to (or lack of benefit from) acetylcholinesterase inhibitors	Mutations
23	M	German	Childhood	–	–	–/–/–/–/	–	+/–/–/–	–/–	+/–/+	–	–	–	+	+	+	+ Tensilon test worsening	T44IA/T44IA
24	F	German	Childhood	–	–	–/–/–/–	–	+/–/–/–	+/–	+/–/+	–	+	–	+	+	+	ND	T44IA/T44IA
25	F	German	Infancy	+	+	–/–/–/–/	–	+/–/–/+	+/–	+/–/+	–	+	+	+	–	+	+	T44IA/T44IA
26	M	Spanish	Childhood	–	–	–/–/–/–	–	+/+/–/–	–/–	+/–/+	–	+	–	+	+	ND	+	Y430S/Y430S
27	F	Spanish	ND	ND	–	–/–/–/–	–	+/+/–/–	+/–	–/–/–	–	+	–	+	+	ND	NT	Y430S/IVSI5+1G>A
28–31	(2) M (2) F	ND	Birth	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	+	+	ND	+	107del2I5/E2I4X 788insC/788insC 1082delC/1082delC E2I4X/E2I4X
32	M	Indian	Birth	+	+	+/+/+/+	ND	+/–/–/+	+/–	+/+/+	+	+	–	+	+	+	+ Negative tensilon test	SI69X/SI69X
33		ND	2	ND	ND	ND	ND	ND	ND	+/ND/+	ND	ND	ND	+	+	ND	+	P59Q/P59Q
34		ND	Birth	ND	ND	ND	ND	ND	ND	+/ND/+	ND	ND	ND	+	+	ND	+	375delT/IVSI5+1G>A
35		ND	Birth	ND	ND	ND	ND	ND	ND	+/ND/+	ND	ND	ND	+	+	ND	+	WI48X/WI48X
36		ND	I	ND	ND	ND	ND	ND	ND	+/ND/+	ND	ND	ND	+	+	ND	+	806insC/806insC
37		ND	Birth	ND	+	ND	ND	ND	ND	ND/ND/–	ND	ND	ND	+	–	ND	+	Q37IX/IVSI5+1G>A
38		ND	I	ND	ND	ND	ND	ND	ND	+/ND/+	ND	ND	ND	+	+	ND	+	R4I0Q/C444Y

+ = yes; – = no; ND = no data; NA = not applicable; the Arabic numeral in brackets denotes the number of the patients.
For the patients with detailed phenotype already published, clinical symptoms not mentioned in the text were presumed to be absent.
Patients 1–5 were published in (Hutchinson *et al*., 1993), Patient 2 also in (Ohno *et al*., 2000) and Patient 4 also in (Ohno *et al*., 1998). Patients 6–13 were published in (Shapira *et al*., 2002). Patients 14–19 were published in (Donger *et al*., 1998). Patients 20 and 21 were published in (Ishigaki *et al*., 2003). Patient 22 was published in (Ohno *et al*., 1999). Patients 23–25 were published in (Müller *et al*., 2004). Patients 26, 27 were published in (Bestue-Cardiel *et al*., 2005). Patients 28–32 were published in (Ohno *et al*., 1998). Patient 32 was published in (Engel *et al*., 1977). Patients 33–38 were published in (Ohno *et al*., 2000).

Table 3 Summary table of the clinical symptoms and laboratory findings in our 22 patients and in the previously published *COLQ*-mutant patients

Clinical symptoms or EMG findings	Published cases	%	Our patients	%
Proximal muscle weakness	22/28	78.5	22/22	100
Lack of objective long-term benefit from AChE inhibitors	36/36	100	19/19	100
Decrement	35/36	97.2	17/19	89.5
Onset at birth or during the first year of life	24/37	64.9	18/22	81.8
Ptosis	12/26	46.2	18/22	81.8
Delayed motor milestones	ND	ND	17/22	77.3
Double CMAP	33/36	91.7	12/18	66.7
Facial weakness	7/25	28	14/22	63.6
Axial muscle weakness/scoliosis	21/32	65.6	14/22	63.6
Myopathic EMG	ND	ND	9/15	60
Ophthalmoparesis	8/27	29.6	13/22	59.1
Respiratory crises	10/29	34.5	10/22	45.5
Neck muscle weakness	8/26	30.8	10/22	45.5
Waddling gait	2/23	8.7	9/21	42.9
Dysphagia and/or chewing difficulties	6/25	24	9/22	40.9
Scapular winging	1/25	4	7/22	31.8
Slow pupillary light response	8/25	32	5/20	25

ND = no sufficient data.

Genotype–phenotype correlations

Genotype–phenotype correlations are difficult in patients with private and/or compound heterozygous mutations. However, mutations Y430S and 1082delC are present homozygously in four and three patients, respectively. The four patients with Y430S mutation are of Spanish descent. The mutation was originally described in a consanguineous Spanish family with six affected individuals (Donger *et al.*, 1998). Recently, two additional *COLQ* patients of Spanish descent carrying Y430S either homozygously or heterozygously have been reported (Bestue-Cardiel *et al.*, 2005). None of our four patients with Y430S presented initial symptoms at birth and all but one had disease onset during preschool age (Table 1). Three of the patients are mildly affected and there is no progression, as it was the case in the previously described patients homozygous for Y430S (Donger *et al.*, 1998; Bestue-Cardiel *et al.*, 2005). In contrast, the disease course was progressive in our Patient 4 leading to considerable disability. Though no haplotype studies were performed, the occurrence of the same mutation in seven unrelated Spanish families, four of them not being consanguineous, suggests a founder effect. A common origin of the molecular defect is supported by the identical intragenic haplotype shared by the mutant chromosomes of our four patients (Supplementary Fig. 1). Our findings, together with the previously reported ones, point that Y430S mutation is generally associated with later onset and a relatively mild 'limb-girdle' phenotype.

The mutation 1082delC was previously described by others (Ohno *et al.*, 1998). We found this mutation homozygously in three patients—two Italian (Patient 18 is from consanguineous family) and one Turkish. The patients presented the myasthenic symptoms at birth or early infancy and have cranial muscle involvement. The Italian patients are moderately to severely affected. In contrast, the Turkish patient is mildly affected.

The mutation T441A was previously reported by us in three German patients (Müller *et al.*, 2004). The identification of the same mutation in one additional German individual, together with the previous haplotype analysis in the other patients, support the hypothesis of a common origin of the mutation (Müller *et al.*, 2004). Similar to the previously reported two siblings, Patient 9 is mildly affected. Interestingly, all four patients with the mutation T441A showed spared cranial muscles and normal pupillary light responses.

Generally, patients carrying two missense mutations seem to be less severely affected than patients with truncating mutations on both alleles. However, there are exceptions to this and, as described above, even patients carrying the same homozygous mutation might display different grade of severity of disease making genotype–phenotype correlation difficult.

No correlation could be established between the location of the mutations in the different domains of *COLQ* and the disease severity. However, patients of our cohort with spared cranial muscles and mild phenotype have homozygous missense mutations in the C-terminal region. No obvious relationship between the amount of asymmetric AChE forms present at the end-plates of patients and the disease phenotype could be established. While, for example, in the mildly affected Spanish patients reported by Donger *et al.*, carrying Y430S asymmetric AChE forms were present at normal level (Donger *et al.*, 1998), absence of most of the asymmetric AChE activity (especially absence of the A12 asymmetric AChE form) was reported for the T441A mutation, which causes a mild phenotype, too, in three out

of four homozygous patients (Müller *et al.*, 2004). Residual A4 and A8 AChE activity present in the patients with T441A might account for the mild phenotype in this case. On the other hand, residual asymmetric AChE is present also in some patients with truncating *COLQ* mutations leading to a severe clinical phenotype (Ohno *et al.*, 2000).

Besides the above-mentioned findings, no clear-cut correlations were established between the disease phenotype and the underlying mutations. Factors other than the type of mutation and the resulting complete or incomplete absence of the asymmetric AChE species at the end-plate are involved in shaping the phenotype. The tendency towards similar phenotypes between siblings [our Patients 7, 8 and 15, 21; the previously published Patients 1 and 2 (Müller *et al.*, 2004)] point to the possible implication of genetic factors in modifying the disease symptoms.

Comparison to other CMS forms

Whereas CMS caused by *COLQ* mutations can usually be distinguished from CMS caused by *CHAT*, *RAPSN* and *CHRNE* (AChR deficiency) mutations by clinical and electrophysiological observations, it shares several features with Slow-channel CMS (SC-CMS) and with limb-girdle CMS caused by mutations in *DOK7* [for a comparison of the most frequent CMS forms, see Table 2 in (Müller *et al.*, 2007*b*)].

Similar to CMS patients with mutations in *DOK7* (Palace *et al.*, 2007; Müller *et al.*, 2007*a*), proximal muscle weakness is frequent in *COLQ*-mutant patients. We found prominent limb-girdle weakness, waddling gait and muscle atrophies in some patients. However, early motor development and eye movements are normal in *DOK7* patients, though there are exceptions (Müller *et al.*, 2007*a*). Five of our patients (Patients 4, 6, 10, 11, 12) demonstrated a phenotype fully compatible with *DOK7* CMS, as there were no delayed motor milestones, the onset was in childhood (Patients 4, 6, 11) and they demonstrated proximal muscle weakness, waddling gait and spared eye movements. The disease progressed in Patient 4 rendering her wheelchair bound, similarly to patients with *DOK7* mutations and there was no repetitive response to single stimulus. Though sinuous gait is considered a hallmark of *DOK7* CMS patients, it was noted in two of our *COLQ*-mutant patients (Patients 6, 12). Moreover, Patients 6 and 11 have fluctuation of symptoms over longer periods of time, which was previously described in *DOK7* CMS patients (Müller *et al.*, 2007*a*). Sequencing of exon 7 of *DOK7* failed to detect mutations in any of these five patients. These cases demonstrate the difficulties that may be encountered in distinguishing *COLQ* and *DOK7* patients, not only because of the similar phenotype, but also because of the missing response to esterase inhibitors treatment in both groups. Interestingly, both CMS groups may respond to ephedrine therapy (Bestue-Cardiel *et al.*, 2005; Beeson *et al.*, 2006; Müller *et al.*, 2007*a*).

Patients 9, 10 and 12 showed proximal muscle weakness and spared facial, bulbar, ocular and respiratory muscles, but the onset was during infancy, there were distal as well as neck weakness in two of them (Patients 9 and 12) and all had double CMAP. In contrast to limb-girdle myasthenia (LGM) with tubular aggregates, none of our *COLQ*-mutant patients had elevated serum CK levels and tubular aggregates were not detected in any of the *COLQ* patients' biopsies. Similar to LGM, a myopathic EMG pattern may be observed in *COLQ*-deficient patients, but double CMAP was never reported in LGM. In contrast to our *COLQ*-mutant patients, patients with LGM and tubular aggregates benefit from long-term esterase inhibitors treatment. Our data, together with the previously published *COLQ* patients with a limb-girdle phenotype (Müller *et al.*, 2004; Bestue-Cardiel *et al.*, 2005) provide further evidence of the genetic heterogeneity of familial LGM.

When a double CMAP in response to single nerve stimulus is observed, mutations in *RAPSN*, *CHAT* and *DOK7* can be ruled out easily. Double CMAP is a characteristic, but not pathognomonic feature of *COLQ*-deficient CMS, because it is also observed in SC-CMS (Engel and Sine, 2005). SC-CMS are usually inherited in autosomal dominant traits, but diagnostic difficulties may be encountered with early onset sporadic or recessive SC-CMS (Croxen *et al.*, 2002), as these patients might also demonstrate respiratory difficulties, poor head control, ptosis and ophthalmoparesis (Milone *et al.*, 1997; Colomer *et al.*, 2006).

Though molecular genetic testing cannot predict the disease course, it is of paramount importance for the establishment of the diagnosis and for initiating the appropriate therapy. The variable clinical manifestations and the possible initial positive response to esterase inhibitors, can lead to considerable diagnostic difficulties. The administration of esterase inhibitors can result in serious complications leading even to fatal outcome in *COLQ*-deficient patients. Mutations in *COLQ* gene arise as the third most common cause of CMS and at a similar frequency with CMS caused by *DOK7* mutations. Therefore, neurologists and paediatricians are likely to face *COLQ*-deficient patients and should recommend molecular genetic testing before initiating esterase inhibitors treatment.

Supplementary material

Supplementary material is available at *Brain* online.

Acknowledgements

We wish to thank the patients and their families for participating in this study. We thank Ursula Klutzny and Mandy Heiliger for technical assistance. We are grateful to Dr Bruno Stober, Dr S. Elmalik, Dr M. Al-Saadi, Dr B. Gervini, Dr P. Lorenzoni and Dr L. Werneck for patient referral. This work was supported by grant from the

Association Francaise contre les Myopathies (AFM). A.A., H.L. and U.S. are members of the German Muscular Dystrophy Network (MD-NET 01GM0601) funded by the German Ministry of Education and Research (BMBF, Bonn, Germany); www.md-net.org. MD-NET is a partner of TREAT-NMD (EC, 6th FP, proposal #036825; www.treat-nmd.eu). J.V. is member of the Spanish Neuromuscular Network funded by Spanish Health Ministry (FIS-PI051622). V.M. receives a BAYHOST fellowship from the Bavarian state. J.S.M. receives a fellowship from the Deutsche Forschungsgemeinschaft (MU2840/1-1).

References

Beeson D, Hantai D, Lochmuller H, Engel AG. 126th International Workshop: congenital myasthenic syndromes, 24–26 September 2004, Naarden, The Netherlands. Neuromuscul Disord 2005; 15: 498–512.

Beeson D, Higuchi O, Palace J, Cossins J, Spearman H, Maxwell S, et al. Dok-7 mutations underlie a neuromuscular junction synaptopathy. Science 2006; 313: 1975–8.

Bestue-Cardiel M, Saenz de Cabezon-Alvarez A, Capablo-Liesa JL, Lopez-Pison J, Pena-Segura JL, Martin-Martinez J, et al. Congenital endplate acetylcholinesterase deficiency responsive to ephedrine. Neurology 2005; 65: 144–6.

Colomer J, Müller JS, Vernet A, Nascimento A, Pons M, Gonzalez V, et al. Long-term improvement of slow-channel congenital myasthenic syndrome with fluoxetine. Neuromuscul Disord 2006; 16: 329–33.

Croxen R, Hatton C, Shelley C, Brydson M, Chauplannaz G, Oosterhuis H, et al. Recessive inheritance and variable penetrance of slow-channel congenital myasthenic syndromes. Neurology 2002; 59: 162–8.

Deprez P, Inestrosa NC, Krejci E. Two different heparin-binding domains in the triple-helical domain of ColQ, the collagen tail subunit of synaptic acetylcholinesterase. J Biol Chem 2003; 278: 23233–42.

Donger C, Krejci E, Serradell AP, Eymard B, Bon S, Nicole S, et al. Mutation in the human acetylcholinesterase-associated collagen gene, COLQ, is responsible for congenital myasthenic syndrome with end-plate acetylcholinesterase deficiency (Type Ic). Am J Hum Genet 1998; 63: 967–75.

Engel AG, Lambert EH, Gomez MR. A new myasthenic syndrome with end-plate acetylcholinesterase deficiency, small nerve terminals, and reduced acetylcholine release. Ann Neurol 1977; 1: 315–30.

Engel AG, Ohno K, Sine SM. Congenital myasthenic syndromes: progress over the past decade. Muscle Nerve 2003a; 27: 4–25.

Engel AG, Ohno K, Sine SM. Sleuthing molecular targets for neurological diseases at the neuromuscular junction. Nat Rev Neurosci 2003b; 4: 339–52.

Engel AG, Sine SM. Current understanding of congenital myasthenic syndromes. Curr Opin Pharmacol 2005; 5: 308–21.

Guerra M, Cartaud A, Cartaud J, Legay C. Acetylcholinesterase and molecular interactions at the neuromuscular junction. Chem Biol Interact 2005; 157–158: 57–61.

Hutchinson DO, Walls TJ, Nakano S, Camp S, Taylor P, Harper CM, et al. Congenital endplate acetylcholinesterase deficiency. Brain 1993; 116 (Pt 3): 633–53.

Ishigaki K, Nicolle D, Krejci E, Leroy JP, Koenig J, Fardeau M, et al. Two novel mutations in the COLQ gene cause endplate acetylcholinesterase deficiency. Neuromuscul Disord 2003; 13: 236–44.

Kimbell LM, Ohno K, Engel AG, Rotundo RL. C-terminal and heparin-binding domains of collagenic tail subunit are both essential for anchoring acetylcholinesterase at the synapse. J Biol Chem 2004; 279: 10997–1005.

Krejci E, Thomine S, Boschetti N, Legay C, Sketelj J, Massoulie J. The mammalian gene of acetylcholinesterase-associated collagen. J Biol Chem 1997; 272: 22840–7.

Milone M, Wang HL, Ohno K, Fukudome T, Pruitt JN, Bren N, et al. Slow-channel myasthenic syndrome caused by enhanced activation, desensitization, and agonist binding affinity attributable to mutation in the M2 domain of the acetylcholine receptor alpha subunit. J Neurosci 1997; 17: 5651–65.

Müller JS, Herczegfalvi A, Vilchez JJ, Colomer J, Bachinski LL, Mihaylova V, et al. Phenotypical spectrum of DOK7 mutations in congenital myasthenic syndromes. Brain 2007a; 130: 1497–506.

Müller JS, Mihaylova V, Abicht A, Lochmüller H. Congenital myasthenic syndromes: spotlight on genetic defects of neuromuscular transmission. Expert Rev Mol Med 2007b; 9: 1–20.

Müller JS, Petrova S, Kiefer R, Stucka R, Konig C, Baumeister SK, et al. Synaptic congenital myasthenic syndrome in three patients due to a novel missense mutation (T441A) of the COLQ gene. Neuropediatrics 2004; 35: 183–9.

Ohno K, Brengman J, Tsujino A, Engel AG. Human endplate acetylcholinesterase deficiency caused by mutations in the collagen-like tail subunit (ColQ) of the asymmetric enzyme. Proc Natl Acad Sci USA 1998; 95: 9654–9.

Ohno K, Brengman JM, Felice KJ, Cornblath DR, Engel AG. Congenital end-plate acetylcholinesterase deficiency caused by a nonsense mutation and an A–>G splice-donor-site mutation at position +3 of the collagenlike-tail-subunit gene (COLQ): how does G at position +3 result in aberrant splicing? Am J Hum Genet 1999; 65: 635–44.

Ohno K, Engel AG, Brengman JM, Shen XM, Heidenreich F, Vincent A, et al. The spectrum of mutations causing end-plate acetylcholinesterase deficiency. Ann Neurol 2000; 47: 162–70.

Palace J, Lashley D, Newsom-Davis J, Cossins J, Maxwell S, Kennett R, et al. Clinical features of the DOK7 neuromuscular junction synaptopathy. Brain 2007; 130: 1507–15.

Schreiner F, Hoppenz M, Klaeren R, Reimann J, Woelfle J. Novel COLQ mutation 950delC in synaptic congenital myasthenic syndrome and symptomatic heterozygous relatives. Neuromuscul Disord 2007; 17: 262–5.

Shapira YA, Sadeh ME, Bergtraum MP, Tsujino A, Ohno K, Shen XM, et al. Three novel COLQ mutations and variation of phenotypic expressivity due to G240X. Neurology 2002; 58: 603–9.

Weschke B, Müller JS, von der Hagen M, Huebner A, Lochmüller H, Spuler S. Partial endplate acetylcholine esterase deficiency due to a novel COLQ-mutation. Neuromusc Disord 2007; submitted.