Available online at www.sciencedirect.com

SciVerse ScienceDirect

Neuromuscular Disorders 22 (2012) 318–324

www.elsevier.com/locate/nmd

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

I. Wargon [a,*], P. Richard [b], T. Kuntzer [c], D. Sternberg [b], S. Nafissi [d], K. Gaudon [b], A. Lebail [b], S. Bauche [e], D. Hantaï [e], E. Fournier [f], B. Eymard [g], T. Stojkovic [g]

[a] *AP-HP, Groupe Hospitalier Pitié-Salpêtrière, Bâtiment Lhermitte, Centre de Recherche de l'Institut du Cerveau et de la Moelle épinière, UMR-S975, Paris, France*

[b] *AP-HP, Groupe Hospitalier Pitié-Salpêtrière, U.F. Cardiogénétique et Myogénétique, Service de Biochimie Métabolique, Paris, France*

[c] *Nerve-Muscle Unit, Neurology Service, Department of Clinical Neurosciences, Lausanne University Hospital (CHUV) and University of Lausanne (UNIL), Lausanne, Switzerland*

[d] *Teheran University of Medical Sciences Shariati Hospital, North Karegar Street, Teheran, Iran*

[e] *AP-HP, Groupe Hospitalier Pitié-Salpêtrière, INSERM U 975, Paris, France*

[f] *AP-HP, Groupe Hospitalier Pitié-Salpêtrière, Département de Neurophysiologie Clinique, Paris, France*

[g] *AP-HP, Groupe Hospitalier Pitié-Salpêtrière, Centre de Référence des maladies Neuromusculaires Paris Est, Paris, France*

Received 24 June 2011; received in revised form 24 August 2011; accepted 2 September 2011

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

* Corresponding author. Address: IM2A, Pavillon Lhermitte, Groupe Hospitalier Pitié-Salpêtrière, 47-83, boulevard de l'Hôpital, 75013 Paris, France. Tel.: +33 (0) 1 42 16 75 37; fax: +33 (0) 1 42 16 75 04.

E-mail address: isabelle.wargon@psl.aphp.fr (I. Wargon).

0960-8966/$ - see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.nmd.2011.09.002

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.