View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Elsevier - Publisher Connector

Am. J. Hum. Genet. 63:967–975, 1998

# Mutation in the Human Acetylcholinesterase-Associated Collagen Gene, *COLQ*, Is Responsible for Congenital Myasthenic Syndrome with End-Plate Acetylcholinesterase Deficiency (Type Ic)

Claire Donger,[1,*] Eric Krejci,[4,*] Adolf Pou Serradell,[5] Bruno Eymard,[1,2] Suzanne Bon,[4] Sophie Nicole,[3] Danielle Chateau,[1] Françoise Gary,[6] Michel Fardeau,[1,2] Jean Massoulié,[4] and Pascale Guicheney[1]

[1]INSERM U153, [2]Institut de Myologie, and [3]INSERM CJF9608, Groupe Hospitalier Pitié-Salpêtrière, and [4]Laboratoire de Neurobiologie Cellulaire et Moléculaire, CNRS URA 1857, Ecole Normale Supérieure, Paris; [5]Servei de Neurologia, Hospital del Mar, Barcelona; and [6]Généthon, Evry, France

## Summary

**Congenital myasthenic syndrome (CMS) with end-plate acetylcholinesterase (AChE) deficiency is a rare autosomal recessive disease, recently classified as CMS type Ic (CMS-Ic). It is characterized by onset in childhood, generalized weakness increased by exertion, refractoriness to anticholinesterase drugs, and morphological abnormalities of the neuromuscular junctions (NMJs). The collagen-tailed form of AChE, which is normally concentrated at NMJs, is composed of catalytic tetramers associated with a specific collagen, COLQ. In CMS-Ic patients, these collagen-tailed forms are often absent. We studied a large family comprising 11 siblings, 6 of whom are affected by a mild form of CMS-Ic. The muscles of the patients contained collagen-tailed AChE. We first excluded the *ACHE* gene (7q22) as potential culprit, by linkage analysis; then we mapped *COLQ* to chromosome 3p24.2. By analyzing 3p24.2 markers located close to the gene, we found that the six affected patients were homozygous for an interval of 14 cM between D3S1597 and D3S2338. We determined the *COLQ* coding sequence and found that the patients present a homozygous missense mutation, Y431S, in the conserved C-terminal domain of COLQ. This mutation is thought to disturb the attachment of collagen-tailed AChE to the NMJ, thus constituting the first genetic defect causing CMS-Ic.**

Received April 20, 1998; accepted for publication August 11, 1998; electronically published September 11, 1998.

Address for correspondence and reprints: Dr. Pascale Guicheney, INSERM U153, Bâtiment Babinski, Groupe Hospitalier Pitié-Salpêtrière, 47 boulevard de l'Hôpital, 75651 Paris Cedex 13, France. E-mail address: pguichen@myologie.infobiogen.fr

* These authors contributed equally to this work.

© 1998 by The American Society of Human Genetics. All rights reserved.
0002-9297/98/6304-0008$02.00

## Introduction

The congenital myasthenic syndromes (CMSs) are a heterogeneous group of diseases caused by genetic defects affecting neuromuscular transmission. They are characterized by weakness and abnormal fatigability on exertion and, in the majority of cases, occur during the first 2 years of life (Bundey 1972; Fenichel 1978). An international workshop supported by the European Neuro Muscular Consortium (ENMC) has classified the CMSs (Middleton 1996) on the basis of their genetic and clinical features. CMS type I corresponds to the autosomal recessive forms: type Ia corresponds to familial infantile myasthenia (MIM 254210), type Ib to limb-girdle myasthenia (MIM 159400), type Ic to acetylcholinesterase (AChE) deficiency, and type Id to acetylcholine receptor deficiency. CMS type II is the autosomal dominant "classic slow channel syndrome" (MIM 601462), which is caused by mutations in the alpha (MIM 100690) or epsilon (MIM 100725) subunits of the acetylcholine receptor.

The CMS-Ic with end-plate AChE deficiency was first described by Engel et al. (1977). It is characterized by onset during childhood, generalized weakness increased by exertion, refractoriness to anticholinesterase drugs, decremental electromyographic response, and morphological abnormalities of the neuromuscular junctions (NMJs) (Engel 1994).

NMJs normally possess a high concentration of collagen-tailed AChE forms, also called "asymmetric," or "A," forms (Massoulié et al. 1993), which may be removed from NMJs by collagenase treatment (Hall and Kelly 1971; Hall 1973). The A4, A8, and A12 forms are defined by the attachment of one, two, and three AChE tetramers, respectively, to the homotrimeric collagen COLQ (Krejci et al. 1997) via a short proline-rich attachment domain (PRAD) (Bon et al. 1997).

Most of the CMS-Ic patients described so far have shown a marked decrease in total muscle AChE, with

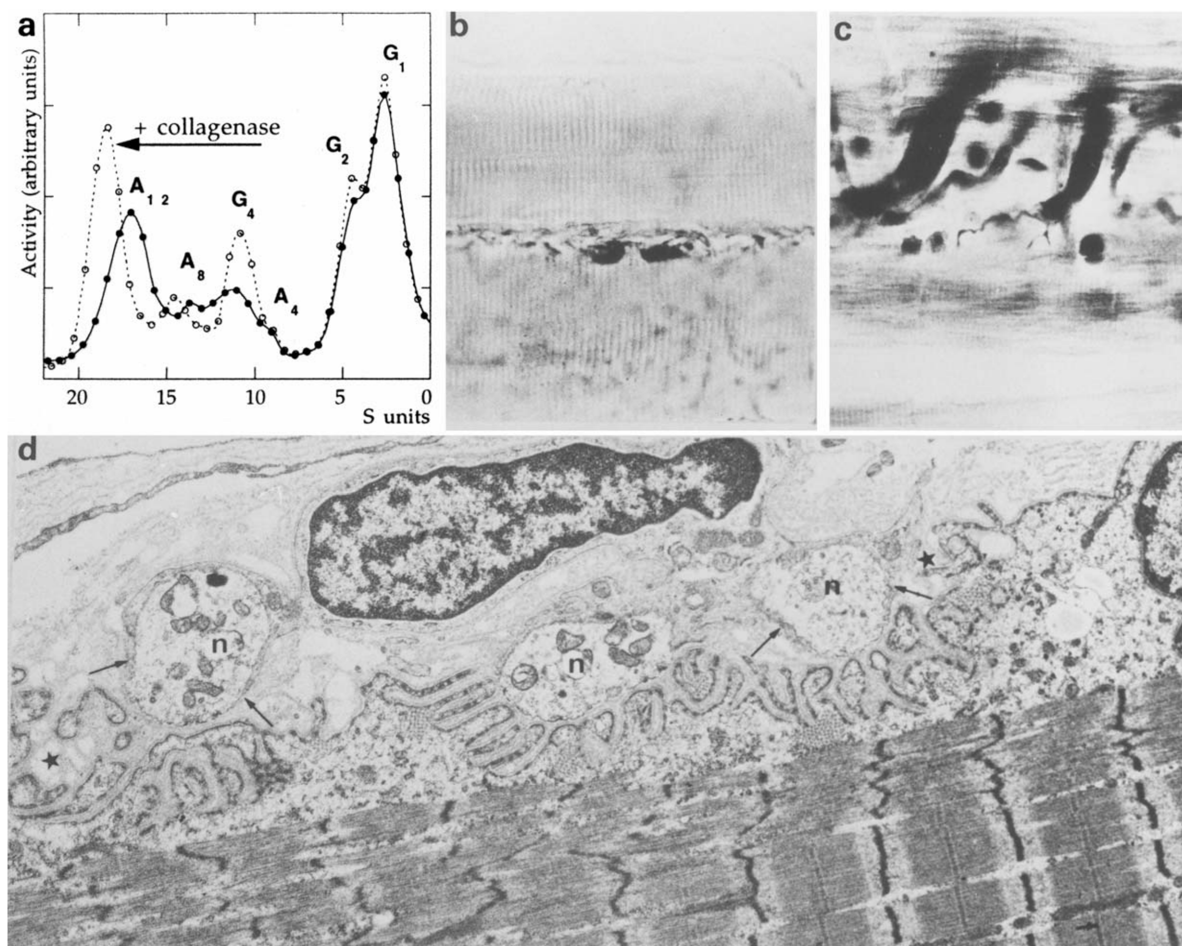


**Figure 1** Morphological and biochemical characterization of NMJs in a CMS-1c patient (II-8). *a,* Sedimentation profiles of AChE in the HSS muscle extract, which revealed the presence of AChE A forms, with a normal sensitivity to collagenase. The sedimentation coefficients were deduced from the position of internal marker proteins, *E. coli* $\beta$-galactosidase (16 S) and alkaline phosphatase (6.1 S). *b,* Histochemical staining of AChE activity at end plates, by the method of Koelle and Friedenwald (1949), which required prolonged incubation (4 h, instead of the 10 min required for normal muscle). In contrast, the density of receptors appeared normal with fluorescein-conjugated $\alpha$-bungarotoxin (not shown). Both methods showed that the end plates were abnormally small ( × 600). *c,* Silver impregnation of nerve terminals on muscle sections, which showed a normal aspect of the terminal-nerve arborization in the end-plate region ( × 600). *d,* Electron microscopy of nerve terminals (n), which appeared to be slightly reduced in size and partly surrounded by Schwann-cell processes (*arrows*), with an apparently normal content of synaptic vesicles. The junctional folds showed marked degenerative changes (*asterisks* [*]). Focal myofibrillar alterations with streaming of the Z lines were seen in the adjacent muscle fibers ( × 7,100).

essentially no collagen-tailed forms (Engel et al. 1977; Jennekens et al. 1992; Hutchinson et al. 1993). Nevertheless, one case with a detectable level of collagen-tailed forms has been reported (Hutchinson et al. 1993).

We here report the analysis of a family with 11 siblings, 6 of whom are affected with a mild form of CMS-Ic. Since childhood, these patients have presented limb weakness and fatigability and single-motor-nerve stimulation–evoked repetitive responses. Analysis of biopsies from two patients revealed that AChE activity at the end plates was abnormally low but that the muscle contained collagen-tailed forms.

Two genes appeared as potential candidates for this disease: *ACHE* (MIM 100740), the AChE gene, located at 7q22 (Ehrlich et al. 1992; Getman et al. 1992), which encodes the catalytic subunits of all molecular species of AChE, and *COLQ,* which encodes the collagen tail of asymmetric forms. In light of the presence of collagen-tailed forms of AChE in the patients' muscles, nonsense mutations and missense mutations were unexpected in these genes' regions directly involved in the formation of the asymmetric forms, but mutations at other sites could not be excluded. We therefore cloned the human *COLQ* gene and determined its genetic localization.

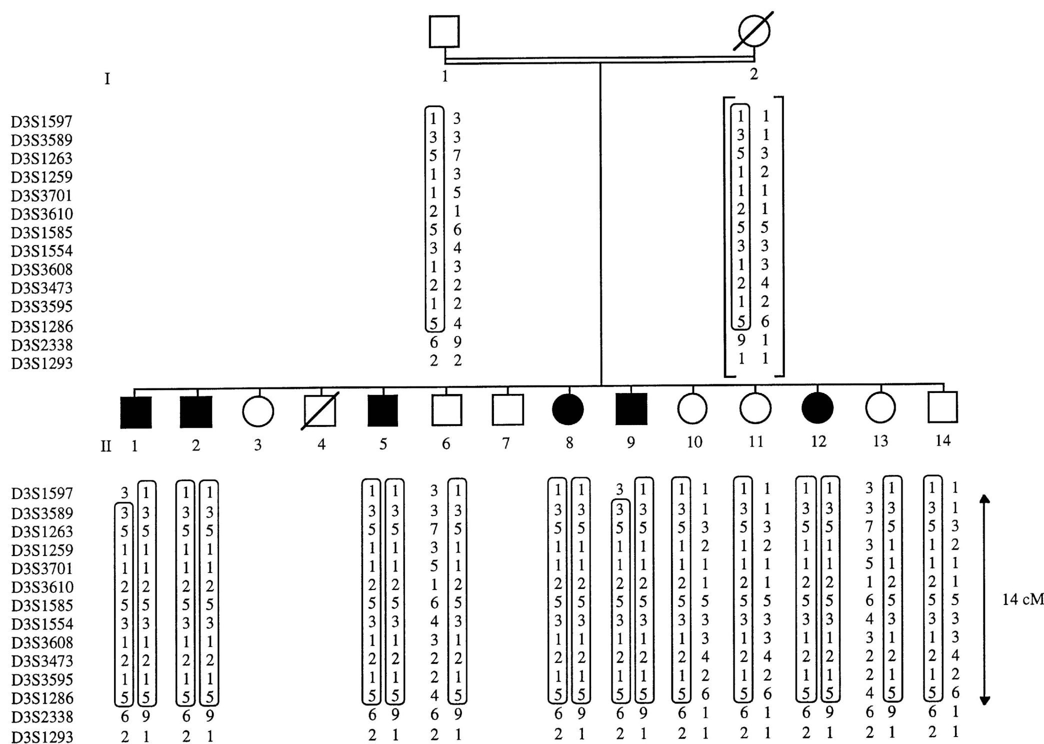


**Figure 2** Pedigree of family 1605. Affected individuals are represented by black dots (for females) and black squares (for males), and unaffected individuals are represented by unblackened circles (for females) and unblackened squares (for males). A diagonal slash through a symbol denotes that the individual is deceased. The haplotypes of chromosome 3p24.2 microsatellite markers are shown below each individual.

Taking advantage of the informativity of the family, we determined the potential involvement of these two genes, by linkage analysis. We found a significant linkage to *COLQ*, and we then identified a first mutation that is likely to cause CMS-Ic.

## Patients and Methods

### *Patients*

We analyzed 11 siblings, ages 36–61 years, from a Spanish family (fig. 1); 6 of them (4 males and 2 females [ages 43–61 years]) were affected with a mild form of CMS-Ic, diagnosed on the basis of the ENMC international workshop criteria (Middleton 1996). The parents were asymptomatic. Blood samples and muscle biopsies were taken after informed consent was obtained. The onset of the myasthenic symptoms occurred at age 6–10 years in all the patients, who complained of great fatigability on exertion, mainly during the 2d decade of life. Two of them presented permanent neck and upper-limb weakness (patient II-8) or diffuse weakness (patient II-9). Electrophysiological studies performed on four patients (patients II-5, II-8, II-9, and II-12) revealed impaired muscle-nerve conduction, with decremental responses at 3 Hz, repetitive responses to single-motor-nerve stimulation, and abnormal jitter. A discrete ptosis and a mild myopathic syndrome were present only in patient II-9. None of the patients had slowed pupil motricity, respiratory distress, or bulbar symptoms. No

## Table 1

**Pairwise LOD Scores between CMS-1c and Chromosome 3p24.2 Markers, for Family 1605**

| | LOD score at Recombination Fraction of | | | | | | |
|---|---|---|---|---|---|---|---|
| Marker | .00 | .01 | .05 | .10 | .20 | .30 | .40 |
| *D3S1597* | −4.86 | −2.08 | −.78 | −.31 | .01 | .09 | .07 |
| *D3S3589* | 2.15 | 2.11 | 1.95 | 1.74 | 1.31 | .86 | .40 |
| *D3S1263* | 4.39 | 4.31 | 3.96 | 3.51 | 2.61 | 1.69 | .77 |
| *D3S1259* | 4.48 | 4.39 | 4.04 | 3.59 | 2.68 | 1.75 | .81 |
| *D3S3701* | 1.83 | 1.80 | 1.66 | 1.49 | 1.12 | .74 | .34 |
| *D3S3610* | 2.97 | 2.90 | 2.63 | 2.30 | 1.68 | 1.08 | .50 |
| *D3S1585* | 1.90 | 1.87 | 1.73 | 1.55 | 1.17 | .77 | .36 |
| *D3S1554* | 1.83 | 1.8 | 1.66 | 1.49 | 1.12 | .74 | .34 |
| *D3S3608* | 3.14 | 3.07 | 2.79 | 2.44 | 1.79 | 1.16 | .55 |
| *D3S3473* | 2.11 | 2.07 | 1.91 | 1.71 | 1.28 | .84 | .39 |
| *D3S3595* | 2.77 | 2.71 | 2.45 | 2.15 | 1.56 | 1.00 | .44 |
| *D3S1286* | 4.49 | 4.40 | 4.05 | 3.60 | 2.69 | 1.76 | .82 |
| *D3S2338* | −2.56 | .15 | 1.00 | 1.26 | 1.18 | .77 | .26 |
| *D3S1293* | −3.87 | −1.26 | −.60 | −.34 | −.13 | −.04 | −.01 |

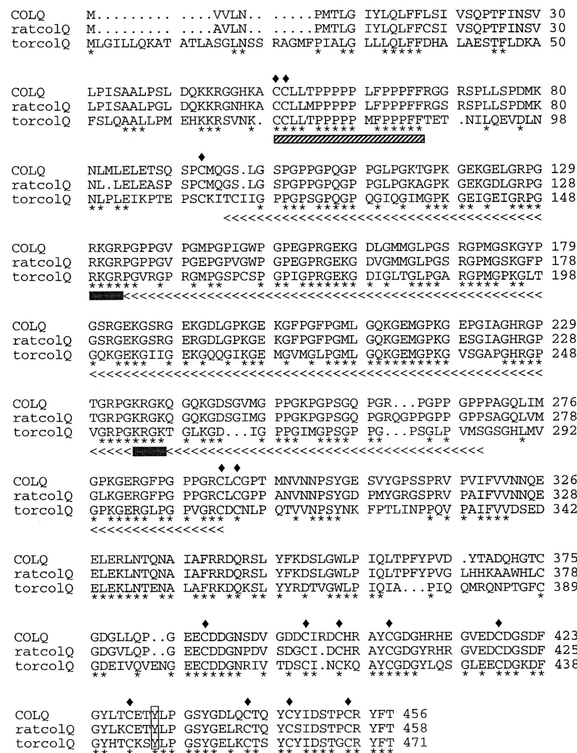


**Figure 3** Alignment of human, rat, and *Torpedo* amino acid sequences of AChE-associated collagen, COLQ. Identical amino acids are denoted by asterisks (*). The hatched box represents the proline-rich attachment domain (PRAD), gray-shaded boxes represent the putative heparan sulfate proteoglycan-binding domains, and the left-facing herringbone symbols (<<<) denote the collagen triple-helical domain, which contains 63 triplets of Gxy in human COLQ. The 15 conserved cysteines are denoted by a blackened diamonds: C51 and C52 in PRAD, which establish disulfide bonds with AChE subunits; C291 and C293, which probably are involved in bonds between the three collagen chains; and the 10 cysteines of the C-terminal domain. The mutated tyrosine is boxed.

anti-AChR antibody was detected, and parenteral administration of anticholinesterase drugs had either no beneficial or even a worsening effect.

### Morphological Analyses

Muscle biopsies were taken from the deltoid muscle, in the end-plate region (patients II-8 and II-9). Cryostat sections of muscle biopsies, taken by means of routine histochemical methods, showed both marked predominance of type I fibers and atrophy of type II fibers. Staining of AChE (Koelle and Friedenwald 1949) and labeling of receptors with fluorescein-conjugated $\alpha$-bungarotoxin showed very small end plates with a reduced subneural apparatus, both on unfixed or on glutaraldehyde-fixed teased muscle fibers. Muscle sections were treated for silver impregnation of the nerve terminals, according to the Gros-Bielchowsky method (Gros and Schultze 1918). Electron microscopy of end plates was performed by conventional methods.

### Biochemical Analysis

AChE was extracted from muscle biopsies (~20 mg), in two steps (Bon et al. 1978). The detergent-soluble fraction (DS) was extracted after both incubation with 650 $\mu$l of detergent buffer (10 mM Tris pH 7.0, 50 mM $MgCl_2$, 1% Triton X-100) with antiproteolitic (2 mM benzamidine, 2 mg bacitracine/ml) and centrifugation for 30 min at 15,000 *g* and 4°C. The pellet was then resuspended in the same buffer, containing 1 M NaCl to extract the high salt–soluble fraction (HSS). AChE molecular forms were analyzed in 5%–20% sucrose gradients in the same buffers. Approximately 45 fractions were collected after centrifugation (16 h at 35,000 *g* in a Beckman SW41 rotor) and were assayed for AChE activity, by the method of Ellman et al. (1961).

The DS extract, corresponding to ~75% of the solubilized activity, contained exclusively globular forms, and the HSS extract, corresponding to ~25% of solubilized activity, contained a significant proportion of A forms, as in the case of normal controls (Carson et al. 1979). Sensitivity to collagenase was studied after incubation of the AChE extracts with 0.01 v of collagenase ABC form III from *Clostridium histologicum* (Advance Biofacture) in 50 mM Tris-HCl pH 8.0 at 25°C.

### Radiation-Hybrid Mapping

The GeneBridge 4 radiation-hybrid panel (Research Genetics) and the following specific intronic primers were used to amplify a 315-bp human *COLQ* DNA fragment, with TCC TGT AAG TAA TGC TAC CAC TGG and TGT TAG ATG TGG ATA GGC CTC C (5′ to 3′), 35 PCR cycles (30 s at 94°C, 30 s at 56°C, and 30 s at 72°C), and "hot start." The products were analyzed by 1.5% agarose gel electrophoresis. The LOD-score calculations were processed by the program RHMAPPER on the Whitehead Institute/MIT center server, with 15 as the LOD score required for demonstration of linkage (Slonim et al. 1997).

### Genotyping

DNA was prepared from blood by means of standard protocols. $(CA)_n$ microsatellite markers were provided by the Généthon human genetic-linkage maps (Dib et al. 1996). Several amplification products from the same DNA sample (80 ng) (Helbling-Leclerc et al. 1995), generated with different primer sets, were pooled and analyzed in a single lane of a 6% denaturating polyacrylamide gel. After they were transferred to Hybond N+ membranes (Amersham), hybridization was performed with a poly(CA) probe.

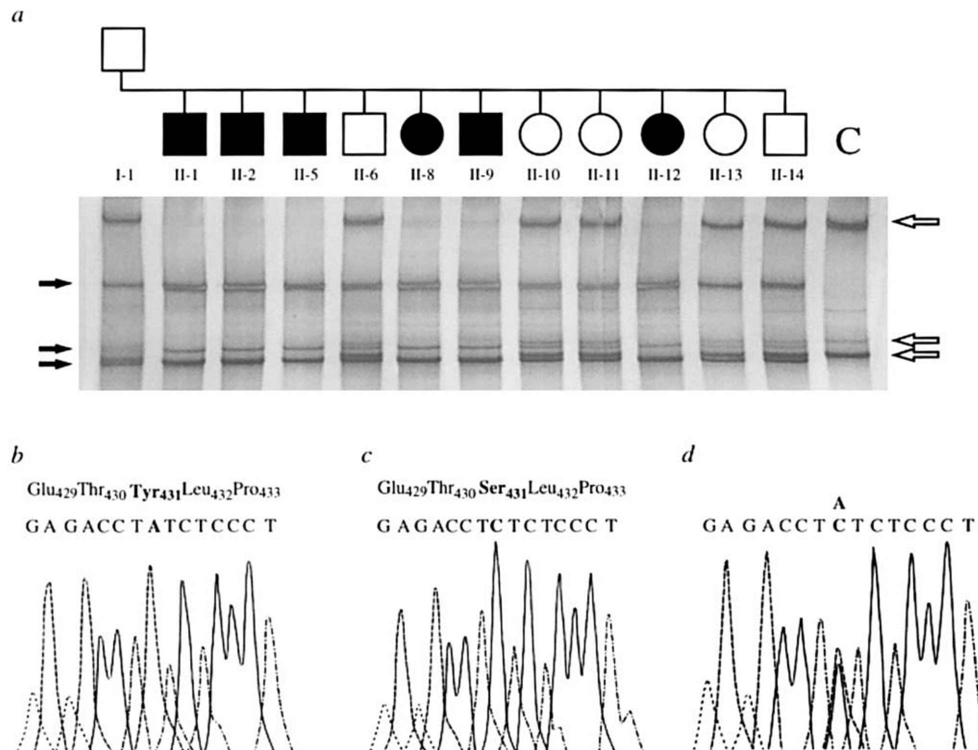


**Figure 4** Identification of the Y431S mutation. *a,* SSCP analysis of PCR products from genomic DNA in 10% polyacrylamide gel, for a control individual (lane C) showing normal migration pattern (*unblackened arrows*), affected individuals (*blackened symbols*) showing a mutant pattern with three characteristic bands (*blackened arrows*), and nonaffected members of the family, displaying fused patterns of mutated and normal alleles (six bands). *b–d,* Detection of a homozygous missense mutation, Y431S, by direct sequencing. Genomic DNA samples were amplified with the primer pair CT3L-CT3R; partial *COLQ* sequences of a healthy control (*b*), an affected individual (*c*), and a heterozygous nonaffected individual (*d*) are shown. The patients present a homozygous A→C substitution at position 1292, which replaces tyrosine 431 by a serine in the C-terminal region of COLQ. Nucleotides are numbered beginning from the ATG of the cDNA sequence.

### *Linkage Analysis*

Linkage analysis was performed by MLINK (LINKAGE version 5.2 package), under the assumption of autosomal recessive inheritance and a partial penetrance, with four arbitrary liability classes: age 0–10 years, age 10–20 years, age 20–40 years, and age >40 years, with penetrances of .40, .60, .80, and .95 respectively. We assumed a disease gene frequency of .001 and equal female and male recombination rates.

### *Human COLQ Sequence*

We screened a human DNA cosmid library with a rat cDNA fragment (891–1420 of rQ1; Genbank accession number AF007583). We mapped and sequenced a genomic fragment containing the exon that encodes the PRAD. We defined PCR primers in this exon and in the C-terminal domain from a human EST, H93653, which was identified, by a Blast search in databanks, on the basis of homology to rat and *Torpedo ColQ* sequences. Using this pair of primers, we amplified a large part of the human *COLQ* coding sequence, both from skeletal muscle biopsies and from lymphoblastoid cell lines. The 5′ part of the cDNA was obtained by 5′ RACE (GibcoBRL, reference number 18374-058) and direct sequencing. We determined the exon-intron organization of the C-terminal noncollagenous domain, by direct sequencing of the cosmid.

### *SSCP Analysis and Direct Sequencing of PCR Products*

SSCP analysis and sequencing of the PCR products were performed as described elsewhere (Helbling-Leclerc et al. 1995). Primers were designed to amplify the 5′ *COLQ* coding sequence from cDNA obtained by reverse transcription (First-Strand cDNA synthesis kit; Pharmacia) of total mRNA prepared from lymphoblastoid cell lines (RNA Plus™; Bioprobe Systems). cDNA amplification required two successive PCRs. The four exons (CT1–CT4) encoding the C-terminal noncollagenous domain were amplified from genomic DNA, with flanking intronic primers. The primers used to amplify exon CT3 were CT3L (5′-ATC CCT TGG GCC GCA TGG T) and CT3R (5′-GCA GGG AGC ACG TGT TGT).

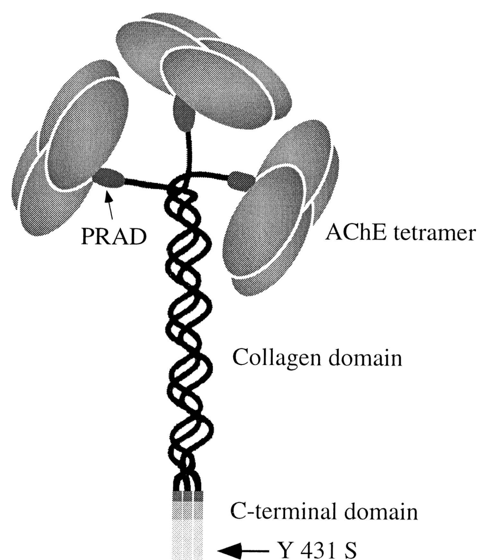


**Figure 5** Schematic representation of the association of tetrameric AChE subunits with collagen Q subunits in the collagen-tailed $A_{12}$ form of AChE. The location of the mutation is indicated by the arrow

## Results

### *Morphological and Biochemical Analyses*

Morphological and biochemical analyses were performed in biopsies of deltoid muscles obtained from patients II-8 and II-9. Histochemical staining revealed that the AChE activity at the end plates was abnormally low, detectable only after several hours of incubation (fig. 1*b*), whereas the density of receptors appeared normal (not shown); in addition, the end plates were much smaller than those in control muscles (fig. 1*b*). Electron microscopy demonstrated that the nerve terminals of the muscles in one of these patients covered only a fraction of the postsynaptic region (fig. 1*d*). The collagen-tailed forms of AChE—the A12 and A8 forms, which sediment at 16 and 14 S, respectively—were present and, in their sedimentation coefficient and sensitivity to collagenase (Carson et al. 1979) (fig. 1*a*), as well as in their aggregation at low ionic strength (not shown), appeared to be identical to those of normal muscle.

### *Linkage Analysis*

Three microsatellite markers—D7S2509, D7S2453, and D7S501—located close to *ACHE* (Collins et al. 1996) were analyzed in all family members; the significant negative LOD scores of −12.38, −9.83 and −9.83, respectively, at a recombination fraction of 0 excluded a defect in the *ACHE* gene. We therefore examined a potential linkage of the disease with *COLQ*. To localize the human gene, we screened a human cosmid library with a rat colQ cDNA probe (Krejci et al. 1997), permitting us to identify and partly sequence a restriction fragment containing the PRAD coding exon.

Using primers derived from this fragment, we mapped the gene on chromosome 3p24.2, between the polymorphic markers D3S1263 and D3S1583, which are separated by a genetic distance of 16 cM. We analyzed 12 microsatellite markers located between these markers and 2 microsatellite markers above D3S1263 (fig. 2). Eleven of these markers were homozygous for the six affected patients (table 1). The deduced at-risk haplotype of the deceased mother happened to be identical to that of the father, suggesting consanguinity. This was not verified; but it would not be surprising, since the parents originated from neighboring villages in Spanish Catalonia. Among the unaffected children, three received the at-risk haplotype from the father and two received it from the mother, making them all heterozygous carriers of the genetic defect (fig. 2). These results indicated that the genetic defect causing the disease in this family was located in a 14-cM interval between D3S1597 and D3S2338. This defect was therefore likely to reside in *COLQ*.

### COLQ *Sequence*

We determined the cDNA sequence of *COLQ* (EMBL accession number AJ225895). It contains 119 nucleotides of the 5′ UTR and 377 nucleotides of the 3′ UTR. The coding sequence (positions 120–1490) is highly homologous to those of rat and *Torpedo*. Human and rat present 89% identity both at the nucleotide level and at the amino acid level, whereas human and *Torpedo* show 64% identity at the nucleotide level and 58% identity at the amino acid level. Alignment of the three deduced peptide sequences shows that nonconserved regions alternate with remarkably well conserved regions, which define potentially functional domains (fig. 3).

### *Detection of the Mutation*

We screened the *COLQ* coding sequence by PCR-SSCP on transcripts from patients' lymphoblastoid cell lines and genomic DNA. The only abnormal SSCP conformers were found in the CT3 exon and cosegregated with the at-risk haplotype (fig. 4). By direct sequencing, we observed an A→C transversion at position 1292, replacing tyrosine 431 (TAT) by a serine (TCT). We did not find this SSCP conformer in 150 unrelated healthy individuals, suggesting that it does reflect a pathological variation.

## Discussion

CMS type Ic is a rare recessive disease, with only six cases reported prior to the present study (Engel et al.

1977; Jennekens et al. 1992; Hutchinson et al. 1993). It is characterized by abnormally low AChE activity in muscles, a reduction of the end-plate size, and abnormal nerve terminals covering only a fraction of the postsynaptic region. In five of these six cases, the collagen-tailed forms of AChE were absent. Indeed, the normal end-plate innervation is probably modulated locally by the AChE activity, since, in CMS-Ic patients, a reduction of end plates was observed, whereas in transgenic *Xenopus,* AChE overexpression promoted neurite outgrowth (Sternfeld et al. 1998).

In the large CMS-Ic family that we have described here, the patients presented not only both a decremental response to repetitive nerve stimulation and repetitive responses evoked by single nerve stimuli, suggesting lack of accumulation of AChE, but also small end plates, abnormal encasement of the presynaptic membrane by Schwann cells, and degeneration of junctional folds, as has been shown elsewhere by electronic microscopy in other CMS-Ic patients (Engel et al. 1977). Nevertheless, biochemical analyses showed the presence of a normal complement of A12 and A8 forms, which, in their sedimentation coefficient, sensitivity to collagenase, and aggregation at low ionic strength, appeared to be identical to those of normal muscle. These patients presented a mild form of the disease, with 6–10 years, instead of 0–2 years, being the age at onset of symptoms, no ophthalmoplegia, and normal pupillary response to light. This delay may be related to modifications of the anchoring of A forms in the NMJ during development. Alternatively, the lack of accumulation of AChE may exert its effect only after a long period. This latter option is supported by the late onset of neuromotor deficiencies in transgenic mice with overexpressed AChE (Andres et al. 1997).

Prior to the present study, the involvement of the AChE gene had been investigated in several CMS-Ic patients with a deficiency in collagen-tailed forms of AChE at the NMJs. This investigation had been performed by means of sequence analysis of the exons that encode the AChE domain to which the tail subunit binds, by Southern analysis of *ACHE* (Hutchinson et al. 1993; Camp et al. 1995) and by a functional approach that investigated the association of the patient enzyme with a foreign tail collagen (Camp et al. 1995). No difference has been found between patients and controls. Since the end plates of the patients in the previous studies lacked the characteristic collagen-tailed forms of AChE, which are associated with the basal lamina, it has been suggested that the defect might reside in either an alteration in a protein involved in promoting the assembly between the catalytic and tail subunits or an altered structure of the tail subunit (Camp et al. 1995). The first hypothesis is not consistent with the fact that coexpression of AChE and ColQ is sufficient to provide oligomerization of collagen-tailed forms in several cell lines (Coussen et al. 1995) and in *Xenopus* oocytes (Krejci et al. 1997). In the present study, we first definitively excluded the AChE gene as a potential culprit by means of linkage analysis; then we analyzed the second hypothesis—the potential involvement of the *COLQ* gene. For this purpose, we localized the human gene on chromosome 3p24.2 and analyzed polymorphic microsatellite markers flanking the gene in all members of the family. We thus found that all patients are homozygous for the genetic interval encompassing *COLQ*. We cloned the human gene and determined the presence of a homozygous sequence variation inducing an amino acid substitution in the patients, which was not found in 300 normal chromosomes even in the heterozygous state. The missense mutation, Y431S, identified in the homozygous state in the six patients and in the heterozygous state in all the unaffected members of the family, changes one of the last amino acids of COLQ.

The protein comprises distinct regions: the N-terminal domain contains the highly conserved PRAD, which binds AChE tetramers (Bon et al. 1997); the central part of the protein is the collagen domain, with its 63 typical Gxy triplets, which participates in the formation of the homotrimeric collagen; and the C-terminal domain contains three conserved regions. The second conserved region plays a role in homotrimeric collagen formation (Krejci et al. 1997), whereas the third conserved region contains nine conserved cysteines, although their exact function is not known. The localization of the mutation in the third conserved region of the C-terminal domain (fig. 5) is consistent with the fact that it does not affect either the formation of the collagen-tailed forms or their sensitivity to collagenase. Ionic interactions of these molecules with polyanionic proteoglycans are currently assumed to be the primary cause for the clustering of AChE in the specialized basal lamina of the NMJ (Bon et al. 1978; Brandan et al. 1985; Rotundo et al. 1997). It has been suggested that these ionic interactions involve two heparin-binding sites, located within the collagen triple-helical region of COLQ (Deprez and Inestrosa 1995) (fig. 2). These sites are not modified by the present mutation, a finding that is in agreement with the normal low salt aggregation of AChE A forms from the patient muscles. Therefore, it appears that the Y431S mutation affects a still unrecognized—but critical—interaction, possibly with a receptor site located either in the extracellular matrix or in the postsynaptic membrane.

Various *COLQ* mutations, missense as nonsense, may compromise several steps of the oligomerization of the A forms or their anchoring in the NMJ and thereby may generate more or less severe pathologies. The most severe forms are associated with a marked decrease in total muscle AChE (Engel et al. 1977; Jennekens et al. 1992; Hutchinson et al. 1993), with essentially no collagen-

tailed forms, but the patients still possess normal levels of globular AChE forms in their erythrocytes (Hutchinson et al. 1993). These pathologies may be caused either by nonsense mutations in the N-terminal part of COLQ or mutations in the PRAD, which would prevent AChE attachment of an AChE tetramer. Mutations in the collagen domain or in the second conserved C-terminal region may also prevent a normal formation of homotrimeric collagen.

In the present article, we have described a family associated with a mild form of CMS-Ic and have reported that the disease is caused by a mutation in the C-terminal part of COLQ. This is the first demonstration of a causal relationship between COLQ mutation and CMS-Ic. Analysis of genes encoding COLQ or interacting proteins in other patients should contribute to elucidation of the molecular basis of human CMS-Ic.

## Acknowledgments

We are indebted to the family members for their invaluable participation. We thank Thomas Melot and Olivier Delattre (INSERM U434) for screening a genomic DNA cosmid library, Dr. Lluis Monserrat (Hospital Vall d'Hebron), Alain Sébille (Hôpital Saint Antoine, Paris) for the electrophysiological analysis of two patients, and Monique Guittard for the histopathological analyses. This work was supported by the Institut National de la Santé et de la Recherche Médicale, the Centre National de la Recherche Scientifique, and the Association Française contre les Myopathies.

## Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

EMBL, http://www.ebi.ac.uk
Genbank, http://www3.ncbi.nlm.nih.gov
Généthon, http://www.genethon.fr
Genome Database, http://gdbwww.gdb.org
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/omim
Whitehead Institute/MIT center, http://www-genome.wi.mit.edu/cgi-bin/contig/rhmapper.pl

## References

Andres C, Beeri R, Friedman A, Lev-Lehman E, Henis S, Timberg R, Shani M, et al (1997) Acetylcholinesterase-transgenic mice display embryonic modulations in spinal cord choline acetyltransferase and neurexin Ibeta gene expression followed by late-onset neuromotor deterioration. Proc Natl Acad Sci USA 94:8173–8178

Bon S, Cartaud J, Massoulié J (1978) The dependence of acetylcholinesterase aggregation at low ionic strength upon a polyanionic component. Eur J Biochem 85:1–14

Bon S, Coussen F, Massoulié J (1997) Quaternary associations of acetylcholinesterase. II. The polyproline attachment domain of the collagen tail. J Biol Chem 272:3016–3021

Brandan E, Maldonado M, Garrido J, Inestrosa NC (1985) Anchorage of collagen-tailed acetylcholinesterase to the extracellular matrix is mediated by heparan sulfate proteoglycans. J Cell Biol 101:985–992

Bundey S (1972) A genetic study of infantile and juvenile myasthenia gravis. J Neurol Neurosurg Psychiatry 35:41–51

Camp S, Bon S, Li Y, Getman DK, Engel AG, Massoulié J, Taylor P (1995) Patients with congenital myasthenia associated with end-plate acetylcholinesterase deficiency show normal sequence, mRNA splicing, and assembly of catalytic subunits. J Clin Invest 95:333–340

Carson S, Bon S, Vigny M, Massoulié J, Fardeau M (1979) Distribution of acetylcholinesterase molecular forms in neural and non-neural sections of human muscle. FEBS Lett 97: 348–352

Collins A, Frézal J, Teague J, Morton NE (1996) A metric map of humans: 23,500 loci in 850 bands. Proc Natl Acad Sci USA 93:14771–14775

Coussen F, Bonnerot C, Massoulié J (1995) Stable expression of acetylcholinesterase and associated collagenic subunits in transfected RBL cell lines: production of GPI-anchored dimers and collagen-tailed forms. Eur J Cell Biol 67:254–260

Deprez PN, Inestrosa NC (1995) Two heparin-binding domains are present on the collagenic tail of asymmetric acetylcholinesterase. J Biol Chem 270:11043–11046

Dib C, Faure S, Fizames C, Samson D, Drouot N, Vignal A, Millasseau P, et al (1996) A comprehensive genetic map of the human genome based on 5,264 microsatellites. Nature 380:152–154

Ehrlich G, Viegas-Pequignot E, Ginzberg D, Sindel L, Soreq H, Zakut H (1992) Mapping the human acetylcholinesterase gene to chromosome 7q22 by fluorescent in situ hybridization coupled with selective PCR amplification from a somatic hybrid cell panel and chromosome-sorted DNA libraries. Genomics 13:1192–1197

Ellman GL, Courtney KD, Andres V, Featherstone RM (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 7:88–95

Engel AG (1994) Myasthenic syndromes. In: Engel AG, Franzini-Armstrong C (eds) Myology, basic and clinical, 2d ed. ed. McGraw-Hill, New York, pp 1798–1835

Engel AG, Lambert EH, Gomez MR (1977) A new myasthenic syndrome with end-plate acetylcholinesterase deficiency, small nerve terminals, and reduced acetylcholine release. Ann Neurol 1:315–330

Fenichel GM (1978) Clinical syndromes of myasthenia in infancy and childhood. Arch Neurol 35:97–103

Getman DK, Eubanks JH, Camp S, Evans GA, Taylor P (1992) The human gene encoding acetylcholinesterase is located on the long arm of chromosome 7. Am J Hum Genet 51: 170–177

Gros V, Schultze O (1918) Neues zur mikroskopischen Untersuchung des Zentralnervensystems. Sitzungsberichte der physicalisch-medizinischen Gesellschaft zu Würzburg 1–5

Hall ZW (1973) Multiple forms of acetylcholinesterase and their distribution in endplate and non-endplate regions of rat diaphragm muscle. J Neurobiol 4:343–361

Hall ZW, Kelly RB (1971) Enzymatic detachment of endplate acetylcholinesterase from muscle. Nat N Biol 232:62–63

Helbling-Leclerc A, Zhang X, Topaloglu H, Cruaud C, Tesson F, Weissenbach J, Tome FM, et al (1995) Mutations in the

laminin alpha 2-chain gene (LAMA2) cause merosin-deficient congenital muscular dystrophy. Nat Genet 11:216–218
Hutchinson DO, Walls TJ, Nakano S, Camp S, Taylor P, Harper CM, Groover RV, et al (1993) Congenital endplate acetylcholinesterase deficiency. Brain 116:633–653
Jennekens FG, Hesselmans LF, Veldman H, Jansen EN, Spaans F, Molenaar PC (1992) Deficiency of acetylcholine receptors in a case of end-plate acetylcholinesterase deficiency: a histochemical investigation. Muscle Nerve 15:63–72
Koelle GB, Friedenwald JS (1949) A histological method for localization of cholinesterase activity. Proc Soc Exp Biol Med 70:617–622
Krejci E, Thomine S, Boschetti N, Legay C, Sketelj J, Massoulié J (1997) The mammalian gene of acetylcholinesterase-associated collagen. J Biol Chem 272:22840–22847
Massoulié J, Pezzementi L, Bon S, Krejci E, Vallette FM (1993) Molecular and cellular biology of cholinesterases. Prog Neurobiol 41:31–91
Middleton LT (1996) Congenital myasthenic syndromes: 34th ENMC international workshop, 10–11 June 1995. Neuromuscul Disord 6:133–136
Rotundo RL, Rossi SG, Anglister L (1997) Transplantation of quail collagen-tailed acetylcholinesterase molecules onto the frog neuromuscular synapse. J Cell Biol 136:367–374
Slonim D, Kruglyak L, Stein L, Lander E (1997) Building human genome maps with radiation hybrids. J Comput Biol 4:487–504
Sternfeld M, Ming G, Song H, Sela K, Timberg R, Poo M, Soreq H (1998) Acetylcholinesterase enhances neurite growth and synapse development through alternative contributions of its hydrolytic capacity, core protein, and variable C termini. J Neurosci 18:1240–1249