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Case report

# Two patients with congenital myasthenic syndrome caused by *COLQ* gene mutations and the consequent ColQ protein defect

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ABSTRACT

*Objective:* To report two cases of congenital myasthenic syndromes (CMS) in a Chinese family with mutations in the *COLQ* gene and to prove the consequence defect of the ColQ protein.
*Method:* Clinical characteristics of the two children from the same family were described. Next-generation sequencing (NGS) and sanger sequencing was performed on the proband and family members. The consequence of the mutation was predicted by 3D protein structure prediction using I-TASSER. The wild type and mutant were transfected to 293T cells, and ColQ protein was detected by Western Blot.
*Results:* The diagnosis of CMS was based on a symptom combination of fatigable muscle weakness, ptosis, scoliosis, and hypotonia, aggravation of muscle weakness after the neostigmine test, and a 46% decrement in repetitive nerve stimulation. A muscle biopsy was performed on the proband, revealing mild variation in the myofiber size. NGS data revealed two compound heterozygous mutations at c.173delC (p.Pro58Hisfs*22) and c.C706T (p.R236X) in the *COLQ* gene, where the former was a novel mutation. A 3D structure prediction showed two truncated ColQ proteins with 78aa and 235aa, respectively. The truncated ColQ protein was proved in 293T cells transfected with c.173delC or c.C706T mutants by Western Blot.
*Conclusions:* The mutations of c.173delC and c.C706T in the *COLQ* gene led to truncated ColQ protein and contributed to the pathogenesis of CMS in this Chinese family.

## 1. Introduction

Congenital myasthenic syndromes (CMSs) are a group of inherited diseases of the neuromuscular junction (NMJ) characterized by fatigable muscle weakness [1,2]. The prevalence of childhood CMSs in the UK has been reported to be 9.2 per million without obvious sex predilection [3]. Yet, a number of cases with late-onset CMSs have been misdiagnosed with other neuromuscular diseases [4]. A

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recent report from Mayo Clinic showed that the misdiagnosis rate reached 94%, and the median diagnostic delay of CMSs was nearly 3 decades in adult patients [5]. With the increasing use of targeted sequencing and NGS, more than 30 genes have been linked to CMSs, of which 12.9% of mutations were found in the *COLQ* gene [6]. *COLQ* gene mutation was first reported by Ohno et al. in 1998 [7]. Thus far, more than 30 mutations in the *COLQ* gene have been proven related to CMSs, including p.P59Q, p.W148*, p.R236X, p.N309S, 1082delC, etc., were identified [2,8,9].

In China, a growing number of CMSs cases have been reported. Also, considerable advances have been made concerning the clinical property and possible mechanisms associated with the *COLQ* gene and other CMS-related gene mutations [10–12]. However, collected data are insufficient to build the database of CMSs in the Chinese population; thus, more patients and cohorts are needed. In this study, we presented a Chinese family with CMS caused by two compound heterozygous mutations located at c.173delC (p.Pro58Hisfs*22) and c.C706T (p.R236X) in the *COLQ* gene. Among which c.173delC is a novel mutation, while c.C706T (p.R236X) was previously reported [13]. To prove the consequence of these two mutations, we predicted the 3D structures of the ColQ peptides using I-TASSER. In addition, we transfected the mutant gene into 293T cells and detected the truncated peptides by Western Blot.

## 2. Materials and methods

### 2.1. Subjects and clinical examinations

The proband was admitted to the Department of Neurology of Shanghai Tongren Hospital (Shanghai, China) in August 2018. All family members underwent detailed assessment for clinical abnormalities, including ptosis, muscle weakness, scoliosis, etc. In this study, the diagnosis of CMS was based on the positive family history, clinical findings during the neonatal period, symptomatic deterioration after the neostigmine test, a decremental compound muscle action potential (CMAP) response at low-frequency (3Hz) repetitive nerve stimulation (LF-RNS), and molecular genetic analysis.

This study was conducted in compliance with the 1964 Declaration of Helsinki. Informed consent, blood samples, and clinical evaluations were obtained from all participants, and the study was approved by the Ethic Committees of Shanghai Tongren Hospital (Ethic 2018-052).

### 2.2. Whole exome sequencing (WES) of the proband

Blood total genomic DNA from the whole family was obtained through the standardized phenol/chloroform extraction method. NGS of the whole exons was performed using standard procedures (Shanghai Raylee Biological Technology Co.LTD). The genomic DNA of the proband was enriched for coding exons with Agilent SureSelect Exon capture Kit and sequenced on the Illumina HiSeq X Ten platform. The depth of the sequencing was 120X. The significant variants were comprehensively evaluated for the allele frequency (1000g, ESP6500, dbSNP, ExAC-East Asian), conservation, predicted pathogenicity, disease association, confirmation with Sanger sequencing, and familial segregation. The pathogenicity of the detected mutations was analyzed using Mutation Taster (http://www.mutationtaster.org/). The targeted pathogenic mutations were verified among the 5 members using Sanger sequencing. The clinical effects of identified variants were classified according to the standards and guidelines published by the American College of Medical Genetics and Genomics (ACMG) [14].

### 2.3. Peptide structure prediction

In order to understand the effect of the compound heterozygous mutation (c.173delC/p.Pro58Hisfs*22 and c.C706T/p.R236X), the protein structure of the wild and mutated ColQ was predicted using I-TASSER (https://zhanglab.ccmb.med.umich.edu/ITASSER-MR/). A 3D protein structure visualization and analysis were performed using PyMOL (Version 1.5, Schrödinger, LLC).

### 2.4. Mutant transfection and Western Blot

To further prove the effect of the mutation, we separately transfected wild-type, c.173delC mutant or c.C706T *COLQ* gene (Gene Create company, Wuhan, China, http://www.genecreate.cn) into approximately $2 \times 10^5$ 293T cells using pcDNA3.1 plasmid by Lipo3000 (ThermoFisher). After 48h, approximately $2 \times 10^6$ cells were collected and lysed with lysis buffer [50 mM Tris–HCl, pH 7.4, 250 mM NaCl, 0.5% Nonidet P-40, plus complete protease inhibitor mixture (Roche Applied Science, 04693116001). The cell lysates were then mixed with 5 × loading buffer (Beyotime, P0015) at a volume ratio of 4:1 and heated to 95° to obtain the final protein samples. After brief vortexing and rotation, protein samples and protein ladder were subjected to SDS-PAGE and then transferred to PVDF membranes. The membranes were first incubated with rabbit anti-human polyclonal antibody of ColQ N terminal (Merck, SAB2107911-100UL) and then with proper HRP-conjugated secondary antibody (CST, 7074S). The immunoreactive bands were detected with ECL plus immunoblotting detection reagents (Amersham Pharmacia Biotech). Molecular weight determination of proteins was established by comparison with the protein ladder (ThermoFisher, 26619).

## 3. Results

### 3.1. Clinical characteristics of the two affected patients

Patient 1 (III-3), the proband, was an 18-month-old boy (Fig. 1A). He weighed 3300 g at birth and was weak crying. He had difficulties with gaining weight. His postural milestones, i.e., his ability to crawl, sit, and walk, were slower compared to his age. Ptosis and head dropping were observed at the age of 6 months. He visited us at the age of 18 months when his physical examination showed bilateral ptosis (Fig. 1B), slow pupillary response, limitation of eye movement, and generalized hypotonia (Fig. 1C). His muscle strength was grade 3/5 on neck flexor, grade 4/5 for the proximal limbs. He had reduced deep tendon reflexes and a positive Gowers sign. A positive chest X-ray showed early signs of thoracic scoliosis (Fig. 1D).

Patient 2 (III-1), a 16-year-old teenager, was born healthy but had difficulty gaining weight. He began to walk at 18 months, and his ability to run and jump was weakened. At 18 months, he suffered from generalized muscle weakness, which was exacerbated by physical activity and relieved by rest breaks. Along with the progression of muscle weakness, he was noticed to suffer from scoliosis from the age of 8 years old. At 16 years old, his scoliosis became so severe that he would suffer dyspnea after activity due to lung compression. Physical examinations were as follows: the patient was thin, 135 cm tall, and 32 kg weight. He had an elongated face (Fig. 1E), bilateral ptosis, slow pupillary response, restricted eye movements, severe thoracic scoliosis (Fig. 1F–H), severe muscle atrophy in his four limbs, and no deep tendon reflexes. His muscle strength was grade 4/5 at neck flexor, 4-/5 at arm abduction, 4-/5 at hip flexor, 4/5 at leg extension, and 4+/5 at distal muscles, based on the Medical Research Council scale (MRC). His ptosis was worsened by the neostigmine diagnostic test (Fig. 2A and B). His pulmonary function exam demonstrated a severe restrictive ventilatory defect due to compression of the lung. A single stimulus in his peroneus showed repetitive compound muscle action potential (CMAP) (Fig. 2C). Repetitive nerve stimulation (3Hz) on the right trapezius showed a 46% decrement in CMAP amplitude (Fig. 2D).

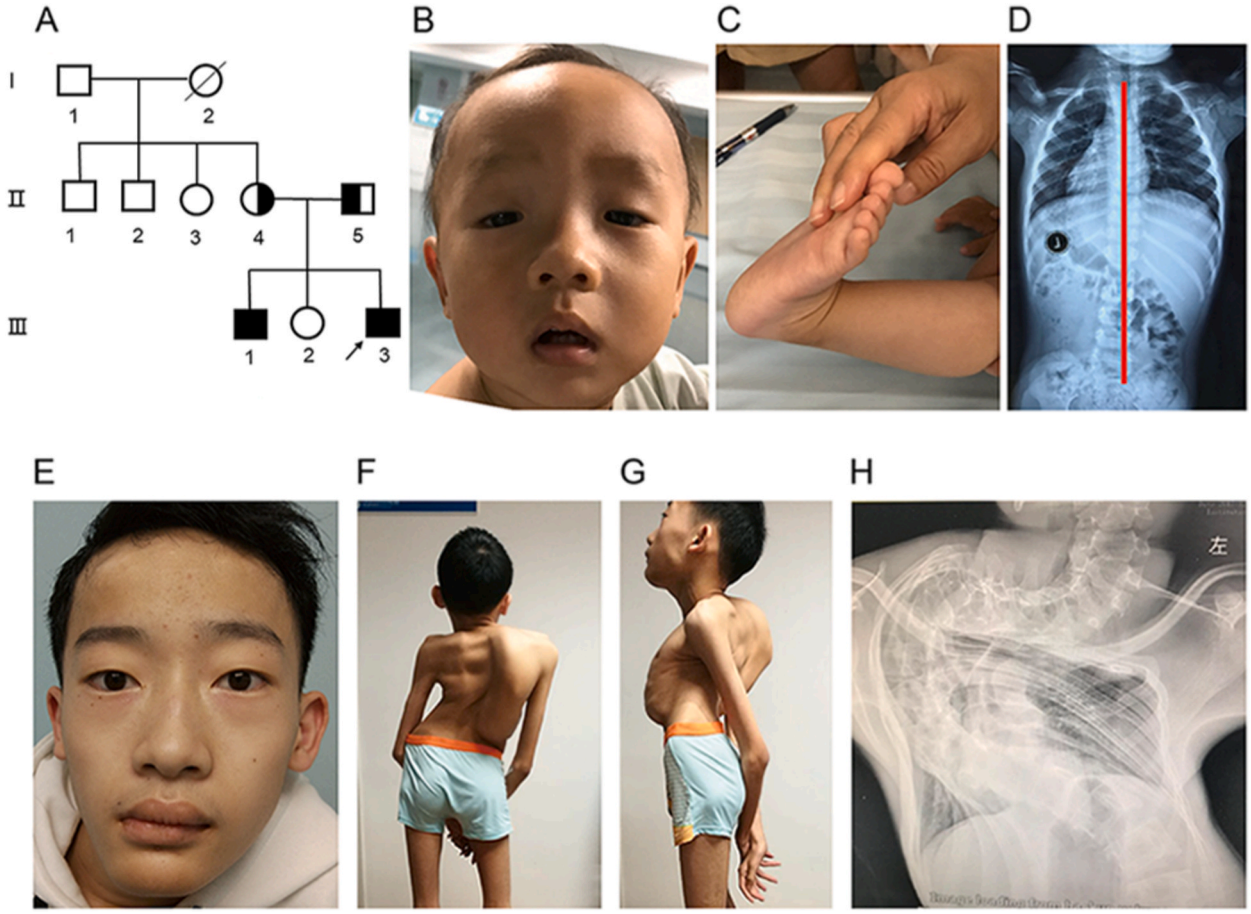


**Fig. 1.** Clinical features of the two patients. **(A)** The family tree of the CMS family revealed two affected male patients and one normal female sibling from the normal parents. The mother and father were carriers of c.173delC and c.C706T mutation, respectively. **(B)** Ptosis and facial muscle palsy in patient 1 (III-3). **(C)** Ankle hypotonia in patient 1. **(D)** Positive chest X-ray image of early scoliosis in patient 1. **(E)** Long and distorted face in patient 2 (III-1). **(F–G)** Scoliosis in patient 2 at 16-year-old. **(H)** Positive X-ray image of severe scoliosis in patient 2 at 16-year-old.

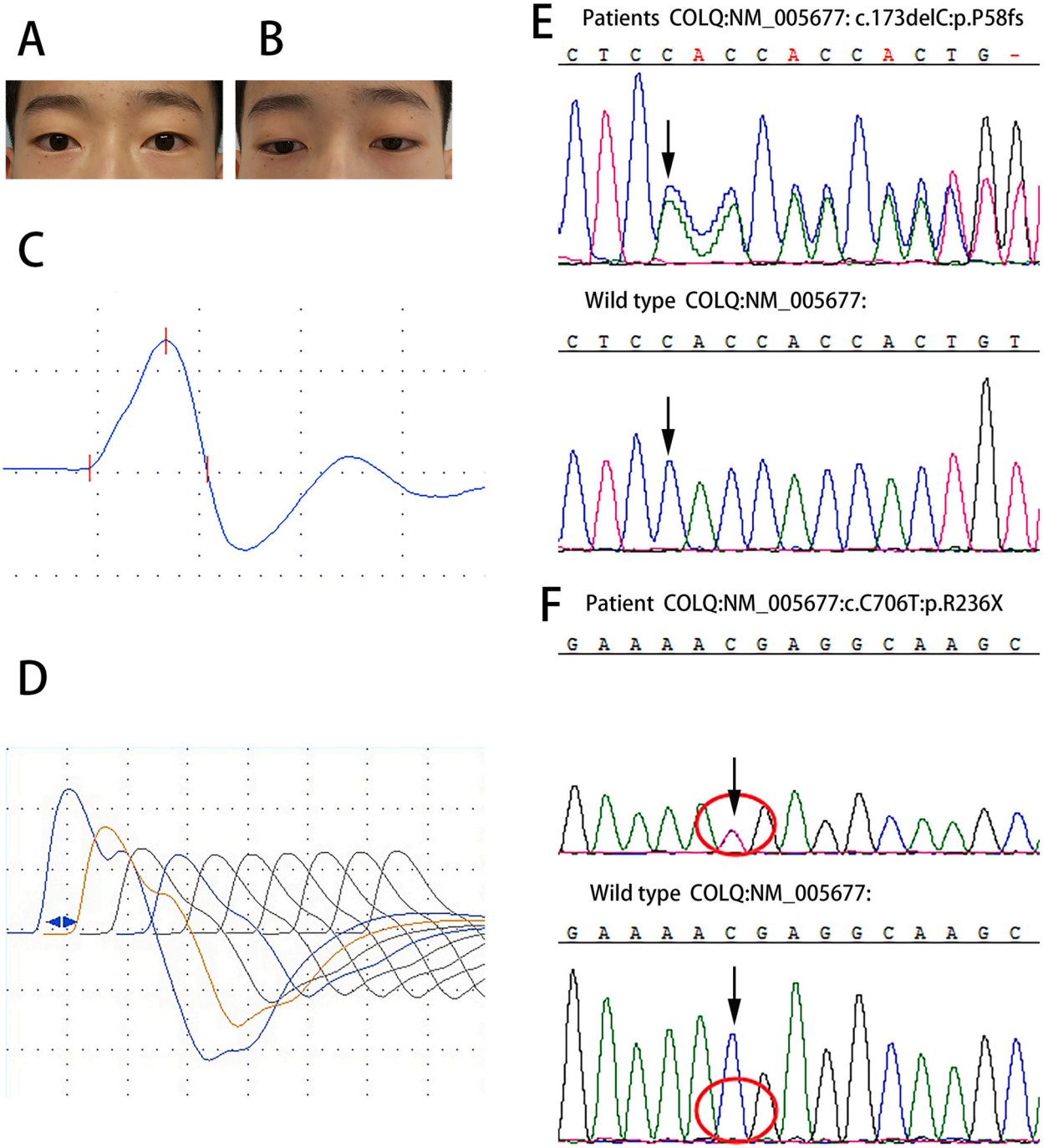


**Fig. 2.** Diagnostic examinations and molecular detections. (A, B) Ptosis of patient 2 was aggravated by neostigmine injection; A) before injection; B) 30 min after injection. **(C)** A single stimulus in the peroneus of patient 2 showed repetitive CMAP. **(D)** Repetitive nerve stimulation (3 Hz) showed a 46% decrement (the 4th wave compared with the 1st one) in CMAP amplitude. **(E, F)** Two heterozygous mutations in the *COLQ* gene revealed by NGS and verified by Sanger sequencing. Mutation at c.173delC (black arrow) is a new mutation that resulted in a frameshift and early stop codon (E); the other mutation c.C706T (black arrow) is a point mutation, which leads to an early stop codon at the antisense allele (F).

### *3.2. Two heterozygous mutations in the COLQ gene*

The WES results revealed two compound heterozygous variants at c.173delC and c.C706T in *the COLQ* (NM_005677) gene in the blood of the proband. The c.173delC mutation was a de novo mutation that resulted in a frameshift and early stop codon (p.Pro58-Hisfs*22) (Fig. 2E). This mutation was predicted as "disease causing" by Mutation Taster and jugged "Pathogenic" (1PVS+1PM+1PP)

according to ACMG guidelines [14]. The other mutation at c.C706T is a previously reported point mutation [9], which leads to an early stop codon at the antisense allele (Fig. 2F). Sanger sequencing of the family showed that the c.173delC mutation was inherited from the unaffected father, and c.C706T mutation was inherited from the unaffected mother. The sick brothers (III-1 and III-3) carried both

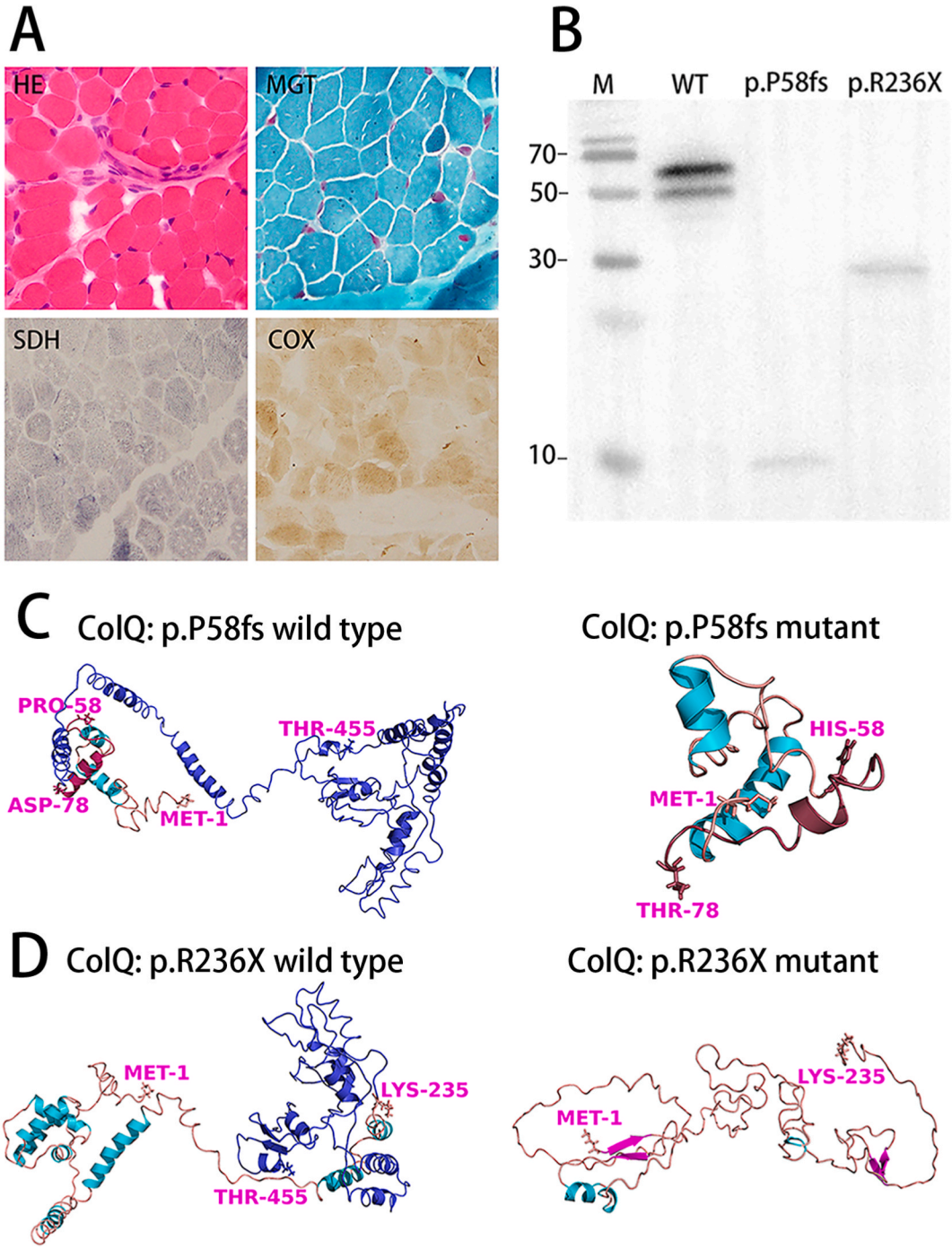


**Fig. 3.** Results of muscle biopsy and verification of the truncated ColQ. **(A)** Muscle biopsy results of the left biceps of patient 1 showed: mild variation in size of the myofiber on HE stain, normal SDH stain, none cox-negative fiber on COX stain, and none RRF and abnormal deposits in modified Gomori trichrome stain. **(B)** Western Blot detected with ColQ antibody in 293T cells transfected with c.173delC or c.C706T showed two truncated proteins of about 9kd and 22kd due to the mutation, while the 293T cells transfected with wild-type (WT) *COLQ* gene showed two bands about 50kd and 55kd ColQ proteins. **(C)** A 3D structure of the mutant ColQ prediction using I-TASSER, and visualized using PyMOL. The mutation of c.173delC was predicted as a truncated ColQ protein of 78aa in length, while the c.C706T mutation as a truncated ColQ protein of 235aa in length.

mutations, and the healthy sister (III-2) carried no mutation.

### 3.3. A 3D protein structure prediction of the mutations

A 3D protein structures of the wild and mutated ColQ protein were predicted using I-TASSER, and visualized using PyMOL. Homozygous mutation of c.173delC generates a truncated ColQ protein, which is 377 amino acid shorter than normal (455 amino acid), while the predicted truncated ColQ protein was 78aa in length (Fig. 3A). The c.C706T mutation leads to a truncated ColQ protein, which is 220 amino acid shorter than normal, while the predicted truncated ColQ protein was 235aa in length (Fig. 3A). Combination of these two heterozygous mutations leads to a truncated ColQ protein without function.

### 3.4. Truncated protein proved by Western Blot

In order to further prove the defect due to these two mutations, the mutant of c.173delC and c.C706T were separately transfected into 293T cells. Western Blot detected with polyclonal ColQ antibody in 293T cells transfected with c.173delC showed a truncated protein of about 9kd, and cells transfected with c.C706T showed a truncated protein of 22kd (Fig. 3B), while the 293T cells transfected with wild type (WT) *COLQ* gene showed two bands of about 50kd and 55kd ColQ proteins (Fig. 3B). These two bands might be explained by protein modification-affected electrophoresis velocity.

## 4. Discussion

The clinical manifestations of CMS are in great variety in clinical manifestations and genetic origins. CMS related to *COLQ* gene mutations are named CMS type-5 (CMS5; MIM #603034), which is responsible for AChE deficiency. Classical phenotype of *COLQ* gene mutation including neonatal manifestations, hypotonia, ptosis, opthalmoparesis, poor crying and suckling, or respiratory insufficiency. AChE inhibitors will aggravate muscle weakness in *COLQ* gene related CMS, whereas treatment with Salbutamol and Ephedrine are effective [1,6,15]. The diagnosis of CMSs from *COLQ* gene mutations is based on the myasthenic symptoms since birth, exertion-induced weakness on examination, negative AChR and MuSK antibodies, and a decremental CMAP response at low-frequency on RNS test [2]. In the present family, the two patients exhibited typical clinical features, including weak cry at birth, failure to gain weight, ptosis, scoliosis, and generalized muscle weakness. The clinical features are summarized in Table 1 (Table 1). The diagnosis of CMS was confirmed by two compound heterozygous mutations at c.173delC and c.C706T in the *COLQ* gene. Our patients conform typical *COLQ* gene related CMS summarized by the literatures [15,16].

To date, more than 30 *COLQ* gene mutations have been reported, including truncate mutations, missense mutations, splicing mutations and transcoding mutations, resulting in downregulation of ColQ protein and its associated enzyme at the basal lamina and thereby leading to lack of ACh degradation. The *COLQ* gene is located on the short arm of chromosome 3, encoding a triple-stranded collagen-like tail (ColQ protein) subunit of asymmetric acetylcholinesterase (AChE). ColQ is responsible for the anchoring of AChE to the endplate of neuromuscular junctions (NMJ) [7]. ColQ includes three domains: proline-rich attachment domain (PRAD), which binds AChE tetramers; the collagen domain, which helps anchoring ColQ to the basal lamina through perlecan; and the C-terminal domain that interacts with muscle-specific tyrosine kinase receptor (MuSK), controlling AChR clustering [8]. Mutations in the PRAD domain are mainly truncated mutation, affecting the assembly of ColQ with AChE to form asymmetric forms of AChE; mutations in the collagen domain are also truncated mutation, impairing the interaction of ColQ with perlecan; and mutations in the C-terminal domain are mainly missense or splicing mutations, prevent the interaction of ColQ with MuSK and basal lamina [7, 17]. Ultimately, mutations in *COLQ* result in endplate AChE deficiency, causing prolonged synaptic action potentials and disrupted AChR clustering [1]. In our family, the c.173delC mutation was in the PRAD region, while the other mutation c.C706T was in the collagen domain. Furthermore, a

**Table 1**
Clinical characteristics of patients in our family.

| | Patient1 | Patient2 |
|---|---|---|
| Gender | Male | Male |
| Age of onset (months) | 3 | 8 |
| Age of diagnosis | 18 months | 16 years |
| Ophthalmoparesis/Ptosis/facial Weakness/Dysphagia | +/++/ ± | ++/+/ ± |
| Proximal/axial/distal/neck weakness | +/++/+/++ | ++/++/+/± |
| slow pupillary response to light | + | + |
| Follow-up (months) | 24 | 24 |
| Pains | – | + |
| Dyspnea/Respiratory crises/Non-invasive ventilation/tracheotomy | −/−/−/− | +/−/-/- |
| Muscle biopsy | muscle fiber variations | ND |
| Clinical status at the end of the follow-up[a] | I | II |
| Acetylcholinesterase inhibitors/ephedrine response | ND/+ | −/+ |

ND: Not done, - Absent, +Present.

[a] Gardner-Medwin and Walton scales: grade 0: normal; grade I: unable to run freely; grade II: difficulty walking on tiptoes; grade III: climbing stairs with banister; grade IV: presence of Gowers' sign; grade V: unable to rise from the floor; grade VI: unable to climb stairs; grade VII: unable to rise from a chair; grade VIII: unable to walk without assistance; grade IX: unable to sit, drink or eat without assistance.

3D structural prediction showed that the two truncated ColQ peptides were 78aa and 235aa, respectively.

To further prove the defect due to these two mutations, we transfected wild type *COLQ* gene, the mutant of c.173delC and c.C706T gene separately into 293T, and detected ColQ peptides using an antibody to ColQ N terminal. The Western Blot of 293T cells transfected with c.173delC and c.C706T showed truncated proteins of about 9kd and 22kd due to the mutation. Compared to the 67kd protein in cells transfected with wild-type *COLQ* gene based on the functional description of Ohno et al. [7], the mutation c.173delC/p.Pro58Hisfs*22 at the PRAD domain may prevent the binding of ColQ to AChE, while the mutation c.C706T/p.R236X at the collagen domain may affect the interaction of ColQ with perlecan. Also, the absence of a C-terminal from both of these mutations makes ColQ protein lose the function of binding to MuSK. To the best of our knowledge, there is no report about the minimum length of the peptide or minimum domains of the protein required to maintain the protein function. However, mutation at the amino acid P59Q next to mutation c.173delC (P58fs) has been verified to prevent the assembly of the asymmetric form of AChE [18].

In conclusion, we demonstrated a novel frameshift mutation at c.173delC. in a Chinese family, which caused CMS combined with c.C706T mutation and confirmed the truncated proteins by 3D structural prediction and Western Blot. This will increase the pathogenic mutation spectrum of CMSs.

## Ethics approval and consent to participate

This study was conducted in compliance with the 1964 Declaration of Helsinki and approved by the Ethics Committee of Shanghai Tongren Hospital. Written informed consent to participate was obtained from the patient's parents.

## Consent for publication

Written informed consent for publication of the case was obtained for the patient's parents. In this study, two cases were under the age of 16.

## Author contribution statement

All authors listed have significantly contributed to the investigation, development and writing of this article.

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## Data availability statement

Data will be made available on request.

## Declaration of interest's statement

The authors declare no competing interests.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.heliyon.2023.e13272.

## References

[1] A.G. Engel, X.M. Shen, D. Selcen, M. Sine, Congenital myasthenic syndromes: pathogenesis, diagnosis, and treatment, Lancet Neurol. 14 (2015) 420–434.
[2] P.B. Shieh, S.J. Oh, Congenital myasthenic syndromes, Neurol. Clin. 36 (2018) 367–378.
[3] J.R. Parr, M.L. Andrew, M. Finnis, D. Beeson, A. Vincent, S. Jayawant, How common is childhood myasthenia? The UK incidence and prevalence of autoimmune and congenital myasthenia, Arch. Dis. Child. 99 (2014) 539–542.
[4] N. Garg, C. Yiannikas, T.A. Hardy, K. Belaya, J. Cheung, D. Beeson, et al., Late presentations of congenital myasthenic syndromes: how many do we miss? Muscle Nerve 54 (2016) 721–727.
[5] J.C. Kao, M. Milone, D. Selcen, X.M. Shen, A.G. Engel, T. Liewluck, Congenital myasthenic syndromes in adult neurology clinic: a long road to diagnosis and therapy, Neurology 91 (2018) e1770–e1777.

[6] A. Abicht, M. Dusl, C. Gallenmuller, V. Guergueltcheva, U. Schara, A. Della Marina, et al., Congenital myasthenic syndromes: achievements and limitations of phenotype-guided gene-after-gene sequencing in diagnostic practice: a study of 680 patients, Hum. Mutat. 33 (2012) 1474–1484.
[7] K. Ohno, J. Brengman, A. Tsujino, A.G. Engel, Human endplate acetylcholinesterase deficiency caused by mutations in the collagen-like tail subunit (ColQ) of the asymmetric enzyme, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 9654–9659.
[8] T. Nakata, M. Ito, Y. Azuma, K. Otsuka, Y. Noguchi, H. Komaki, et al., Mutations in the C-terminal domain of ColQ in endplate acetylcholinesterase deficiency compromise ColQ-MuSK interaction, Hum. Mutat. 34 (2013) 997–1004.
[9] A. Abicht, J.S. Muller, H. Lochmuller, Congenital myasthenic syndromes overview, in: M.P. Adam, D.B. Everman, G.M. Mirzaa, R.A. Pagon, S.E. Wallace, L.J. H. Bean, K.W. Gripp, A. Amemiya (Eds.), GeneReviews® [Internet], University of Washington, Seattle, Seattle (WA), 2003 May 9, pp. 1993–2022.
[10] X. Luo, C. Wang, L. Lin, F. Yuan, S. Wang, Y. Wang, et al., Mechanisms of congenital myasthenia caused by three mutations in the COLQ gene, Front Pediatr 9 (2021), 679342.
[11] Q.L. Zhang, M.J. Xu, T.L. Wang, Z.Q. Zhu, F. Lai, X.C. Zheng, Newly discovered COLQ gene mutation and its clinical features in patients with acetyl cholinesterase deficiency, J. Integr. Neurosci. 17 (2018) 439–446.
[12] Y. Zhao, Y. Li, Y. Bian, S. Yao, P. Liu, M. Yu, et al., Congenital myasthenic syndrome in China: genetic and myopathological characterization, Ann. Clin. Transl. Neurol 8 (4) (2021) 898–907.
[13] U. Yiş, K. Becker, S.H. Kurul, G. Uyanik, E. Bayram, G. Haliloğlu, et al., Genetic landscape of congenital myasthenic syndromes from Turkey: novel mutations and clinical insights, J. Child Neurol. 32 (8) (2017) 759–765.
[14] S. Richards, N. Aziz, S. Bale, D. Bick, S. Das, J. Gastier-Foster, et al., Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of medical genetics and genomics and the association for molecular pathology, Genet. Med. 17 (2015) 405–424.
[15] E.P. Estephan, A.A. Zambon, R. Thompson, K. Polavarapu, D. Jomaa, A. Topf, et al., Congenital myasthenic syndrome: correlation between clinical features and molecular diagnosis, Eur. J. Neurol. 29 (3) (2022) 833–842.
[16] C. Farmakidis, M. Pasnoor, R.J. Barohn, M.M. Dimachkie, Congenital myasthenic syndromes: a clinical and treatment approach, Curr. Treat. Options Neurol. 20 (2018) 36.
[17] J. Arredondo, M. Lara, F. Ng, D.A. Gochez, D.C. Lee, S.P. Logia, et al., COOH-terminal collagen Q (COLQ) mutants causing human deficiency of endplate acetylcholinesterase impair the interaction of ColQ with proteins of the basal lamina, Hum. Genet. 133 (2014) 599–616.
[18] H. Dvir, M. Harel, S. Bon, W.Q. Liu, M. Vidal, C. Garbay, et al., The synaptic acetylcholinesterase tetramer assembles around a polyproline II helix, EMBO J. 23 (2004) 4394–4405.