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ORIGINAL COMMUNICATION

Congenital myasthenic syndrome caused by novel *COL13A1* mutations

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Abstract

Collagen XIII is a non-fibrillar transmembrane collagen which has been long recognized for its critical role in synaptic maturation of the neuromuscular junction. More recently, biallelic *COL13A1* loss-of-function mutations were identified in three patients with congenital myasthenic syndrome (CMS), a rare inherited condition with defective neuromuscular transmission, causing abnormal fatigability and fluctuating muscle weakness and often successfully treated with acetylcholinesterase inhibitors. Here we report six additional CMS patients from three unrelated families with previously unreported homozygous *COL13A1* loss-of-function mutations (p.Tyr216*, p.Glu543fs and p.Thr629fs). The phenotype of our cases was similar to the previously reported patients including respiratory distress and severe dysphagia at birth that often resolved or improved in the first days or weeks of life. All individuals had prominent eyelid ptosis with only minor ophthalmoparesis as well as generalized muscle weakness, predominantly affecting facial, bulbar, respiratory and axial muscles. Response to acetylcholinesterase inhibitor treatment was generally negative while salbutamol proved beneficial. Our data further support the causality of *COL13A1* variants for CMS and suggest that this type of CMS might be clinically homogenous and requires alternative pharmacological therapy.

Keywords *COL13A1* · Collagen type XIII alpha 1 chain · Autosomal recessive · Congenital myasthenic syndrome · Neuromuscular junction

Introduction

Congenital myasthenic syndromes (CMS) are rare inherited conditions affecting neuromuscular transmission resulting in abnormal fatigability and fluctuating muscle weakness [1]. Some forms of CMS may lead to generalized paralysis and respiratory failure, and if untreated, can be life threatening.

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CMS is genetically heterogeneous with disease causing mutations found in more than 20 different genes. Depending on the underlying genetic defect, treatment with acetylcholinesterase (AChE) inhibitors or other substances is beneficial [10], making CMS one of the seldom examples of treatable inherited disorders and highlighting the need for a precise molecular genetic diagnosis. Recently, homozygous loss-of-function variants in the *COL13A1* gene have been reported as a new cause of CMS (CMS type 19, OMIM #616720) [6]. Affected individuals had feeding difficulties, respiratory compromise (with episodes of deterioration in respiratory function in some cases), facial and axial weakness as well as prominent eyelid ptosis but no or only mild ophthalmoparesis. Treatment with AChE inhibitors was not effective. However, conclusions concerning the phenotypic spectrum are still limited, as only three cases have been reported so far.

Materials and methods

All participants or their legal guardians provided informed consent for molecular genetic studies and the LMU Munich ethics committee approved the study (084/00). The study has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Whole-exome sequencing (WES) in the probands and further work-up of WES results were performed as described in Appendix S1, Supporting Information.

Results

We report on six affected individuals from three consanguineous families (Fig. 1a; Table 1). In all individuals, the disease manifested at birth after uneventful pregnancies. Presenting symptoms were neonatal respiratory distress and dysphagia, which often resolved or improved within days or weeks, generalized muscular hypotonia and eyelid ptosis. Four individuals reached their early motor milestones late. Disease course was variable, with stable or even improving symptoms (particularly of respiratory and bulbar functions) in some cases while others experienced progressive decline of respiratory function when they grew older (A-II:2, C-II:3). Patients had prominent, often non-fluctuating eyelid ptosis (Fig. 1b) but no relevant ophthalmoparesis. Weakness was most severe in facial, bulbar, respiratory and axial muscles (Fig. 1b) while limb muscles were less affected. All but one individual showed abnormalities of the spine including spinal rigidity, scoliosis and kyphosis. Mild facial dysmorphisms (low-set ears, micrognathia and high-arched palate) and pectus carinatum were additional findings. None of the patients had congenital contractures; there was no intellectual disability except for patient B-II:1 who had moderate cognitive delay. Patient B-II:1 suffered from an additional autosomal recessive condition, congenital insensitivity to pain with anhidrosis (CIPA), caused by a homozygous *NTRK1* mutation. Genetic and clinical findings have been reported elsewhere [9]. Patient B-II:1 died at the age of 20 years due to a choking episode. Treatment with oral pyridostigmine bromide (A-II:2) or a combination of pyridostigmine bromide and 3,4-diaminopyridine (B-II:2) had no clearly beneficial effect. Application of salbutamol (0.6 mg/kg/day) in A-II:2 improved posture of the body and head, walking ability and overall endurance. Similarly, oral salbutamol also improved respiratory function and abated respiratory crises in case B-II:2.

Levels of serum creatine kinase (CK) were normal. Standard electromyography and nerve conduction studies yielded normal results in three individuals while one case (B-II:1) had mild myopathic changes in proximal muscles. Whenever performed (three cases), repetitive nerve stimulation (RNS) at 2–3 Hz showed decrement of the compound muscle action potential (CMAP) amplitudes in the corresponding muscles (facial and distal and proximal extremities). A muscle biopsy from the deltoid muscle of patient A-II:2 showed mild myopathic changes (increased variability of fiber sizes, slight increase of endomysial connective tissue, Fig. 1c). Type I fiber predominance was noted in a muscle specimen from patient B-II:2 while muscle biopsies obtained from cases B-II:1 and C-II:2 revealed no distinct abnormalities.

Several known CMS genes and genes associated with other neuromuscular disorders (family A: *DOK7* (exon 7), *COLQ, FKRP*, and *D4Z4* repeat; family B: *RAPSN, DOK7* (exon 7), *CHAT, CHRND, MUSK*, and *GFPT1*; family C: *RAPSN* (exon 2) and *DOK7* (exon 7)) were excluded as part of clinical genetic testing. We subsequently performed WES using DNA samples of one affected individual from each family. Using an in-house pipeline [2], variants were filtered for ultra-rare, biallelic non-synonymous variants. Considering parental consanguinity, we focused on homozygous variants among which loss-of-function variants in the *COL13A1* gene represented the most plausible candidates (Table S1, Supporting Information). No pathogenic mutation was identified in the remaining 25 genes, which are associated with CMS [1]. Variants were confirmed by Sanger sequencing of the respective exons of the *COL13A1* gene (Fig. 1A) and segregation with the phenotype was tested in available family members.

Discussion

Collagen type XIII alpha 1 chain (COL13A1) has been first implicated in regulating the maturation of the neuromuscular junction in mouse models more than 15 years ago [4]

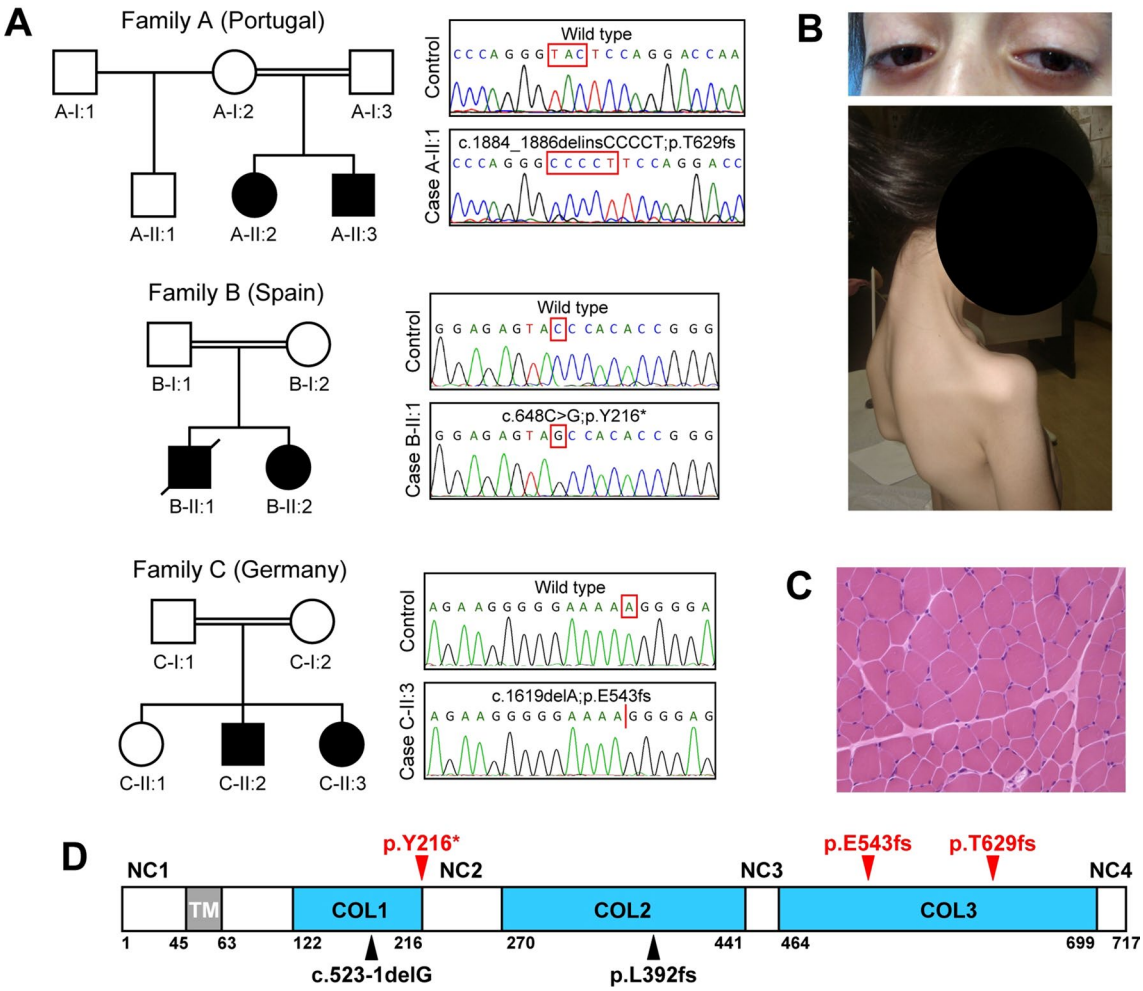

Fig. 1 *COL13A1*-associated CMS. **a** Pedigrees of three CMS families with affected individuals carrying biallelic *COL13A1* variants. Chromatograms display the wild-type reference sequence and the patients' genotypes. **b** Patient A-II:2: bilateral eyelid ptosis, impaired neck extension, scapulae alatae and slender muscle build. **c** Patient A-II:2: muscle biopsy from the deltoid muscle (biopsy taken at age 8 years) displaying abnormal variation in fiber size and slight increase of endomysial connective tissue. **d** Overview of *COL13A1* mutations found in this and an earlier study [6]. Variants reported in this study are colored in red. *COL1-3* collagenous domains, *NC1-4* non-collagenous domains, *TM* transmembrane domain. Numbers indicate amino acid residues composing each domain

and its function in the postsynaptic membrane and synaptic basement membrane has been studied extensively since then [3, 5]. COL13A1 has been postulated as a potential CMS-associated protein earlier [7], and this has been confirmed recently when biallelic *COL13A1* loss-of-function mutations were identified in two CMS families [6]. The three affected individuals in these families presented with prominent eyelid ptosis, respiratory compromise, bulbar dysfunction and no response to AChE inhibitor medication. The clinical picture of the six new *COL13A1* patients reported here was strikingly similar, suggesting that *COL13A1* mutations might be associated with a relatively invariant phenotype.

The novel *COL13A1* mutations p.Tyr216*, p.Glu543fs and p.Thr629fs identified in our patients from three unrelated families have not yet been reported in CMS families. However, pathogenicity is supported by the predicted loss-of-function effect of these mutants, their absence from databases on normal variation of the human genome (gnomAD, EVS, dbSNP), and their segregation with disease status in the respective families. Because the mutations did not affect the terminal exon of the gene, their most likely consequence was nonsense-mediated mRNA decay resulting in no COL13A1 protein. A similar effect is expected for the previously reported *COL13A1* mutations [6]. Alternatively, the mRNA could be translated into a truncated protein lacking one or more intact C-terminal collagenous domains (COL2, COL3) (Fig. 1d). Especially, COL3 is considered critical for protein function as it is the largest and outermost of the collagenous domains [3].

Table 1 Clinical features of individuals with biallelic *COL13A1* mutations

Reference	This report						Logan et al. (2015) [6]		
Family	Family A		Family B		Family C		Family 1	Family 2	
Ethnicity	Portuguese		Spanish		German		White European	Indian subcontinent	
Consanguinity	Yes		Yes		Yes		No	Yes	
Mutations	p.T629fs (hmz.)		p.Y216* (hmz.)		p.E543fs (hmz.)		p.L392fs (hmz.)	c.523-1delG (hmz.)	
Individual	A-II:2	A-II:3	B-II:1	B-II:2	C-II:2	C-II:3	II:1	II:1	II:2
Sex/age	F/19 years	M/11 years	M/died at 20 years	F/22 years	M/26 years	F/25 years	F/2 years	M/27 years	F/died at 8 years
Age at assessment	18 years	10 years	8 years	20 years	7 years	13 years	5 months	25 years	5 years
Pregnancy	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Age of onset	Birth	Birth	Birth	Birth	Birth	Birth	Birth	Infancy	Birth
Manifesting symptoms	Hypotonia, ptosis, respiratory distress[a]	Hypotonia, ptosis	Hypotonia, respiratory distress[a], dysphagia[a]	Hypotonia, ptosis, respiratory distress, dysphagia	Hypotonia, respiratory distress, dysphagia	Hypotonia, respiratory distress[a], dysphagia	Hypotonia, respiratory distress[a], dysphagia	Ptosis, respiratory distress[a], dysphagia[a]	Respiratory distress[a], dysphagia[a]
Motor milestones	Normal	Normal	Delayed	Delayed	Delayed	Delayed	Delayed	Normal	Delayed
Cognitive deficits	No	No	Yes	No	No	No	Not assessed due to young age	Yes	Not reported
Disease course	Progressive (worsening respiratory function)	Rather stable	Rather stable	Rather stable	Stable, improvement	Progressive (worsening respiratory function)	Stable, improvement	Stable, improvement	Progressive, died from respiratory failure
Ptosis	Yes (constant)	Yes (constant)	Yes	Yes (constant)	Yes	Yes (constant)	Yes (constant)	Yes (constant)	Yes
Ophthalmoparesis	No	No	No	No	No data	No	No	No	Yes (mild)
Facial weakness	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes
Bulbar weakness	Yes (nasal speech)	Not reported	Yes (nasal speech)	Yes (dysphagia)	Yes (dysphagia, gastric tube-fed)	Yes (dysphagia)	Yes	No	Yes
Axial weakness	Yes	Yes	Yes	Yes	Yes (almost no head control)	Yes	Yes	Yes	Yes
Weakness UL	P > D	P > D	P > D	P > D	P > D	P > D	P = D	No	P = D
Weakness LL	P > D	P > D	P > D	P > D	P > D	P > D	P = D	P	P = D
Respiratory compromise	Yes (non-invasive ventilation)	Yes	No	Yes (non-invasive ventilation)	Yes (ventilation, at night, tracheostoma	Yes (vital capacity 27%)	Yes	Yes	Yes

Table 1 (continued)

Reference	This report						Logan et al. (2015) [6]		
Family	Family A		Family B		Family C		Family 1	Family 2	
Ethnicity	Portuguese		Spanish		German		White European	Indian subcontinent	
Consanguinity	Yes		Yes		Yes		No	Yes	
Mutations	p.T629fs (hmz.)		p.Y216* (hmz.)		p.E543fs (hmz.)		p.L392fs (hmz.)	c.523-1delG (hmz.)	
Individual	A-II:2	A-II:3	B-II:1	B-II:2	C-II:2	C-II:3	II:1	II:1	II:2
Exacerbations	No	No	No	Yes (respiratory crises)	Not reported	No	Yes (respiratory crises)	Not reported	Not reported
Dysmorphic features	Micrognathia	Micrognathia	Micrognathia, high-arched palate, pectus carinatum	Micrognathia, high-arched palate, pectus carinatum	Not reported	Micrognathia, high-arched palate	Micrognathia, high-arched palate, pectus carinatum	Micrognathia, high-arched palate, pectus carinatum	Micrognathia, high-arched palate, pectus carinatum
Abnormalities of the spine	Scoliosis	Kyphosis, scoliosis	No	Scoliosis	Scoliosis	Scoliosis	No	Spinal rigidity	Spinal rigidity, kyphosis
Scapulae alatae	Yes	Yes	Not reported	Not reported	Not reported	Yes	Not reported	Not reported	Not reported
Contractures	No	No	No	No	Not reported	No	No	No	No
CK levels	Normal	Normal	Normal	Normal	Not reported	Normal	Normal	Not reported	Not reported
Standard EMG	Normal	Not done	Mild myopathic pattern	Normal	Not reported	Normal	Not reported	Not reported	Not reported
RNS decremental response/muscles	Yes/distal and proximal	Not done	Yes/distal, proximal and facial	Yes/distal, proximal and facial	Not done	Not done	Yes/hand	Yes/hand	Not reported
Muscle biopsy	Myopathic changes (mild)	Not done	Unspecific changes (mild)	Type I fiber predominance	Unspecific changes (mild)	Not done	Myopathic (mild)	Not done	Not reported
Therapy/effect	Pyridostigmine/none; salbutamol/positive	No medication	No medication	Pyridostigmine + 3,4-DAP/none; salbutamol/positive	No medication	No medication	Pyridostigmine/none; 3,4-DAP + salbutamol/positive	Pyridostigmine/none	No medication

[a]Resolved or markedly improved in the first days or weeks of life

3,4-DAP 3,4-diaminopyridine; *CK* serum creatine kinase; *D* distal; *EMG* electromyography; *F* female; *hmz.* homozygous; *LL* lower limbs; *M* male; *P* proximal; *RNS* repetitive nerve stimulation; *UL* upper limbs

As observed in the study by Logan et al. [6], we also found that AChE inhibition, which is effective in many other CMS types, is not beneficial in patients with *COL13A1*-associated CMS. One case in the earlier report showed a positive response to combined treatment with salbutamol and 3,4-diaminopyridine. Salbutamol alone was tried in two individuals from our series (A-II:2, B-II:2) and showed clear positive effects, suggesting that salbutamol monotherapy might be effective as well in CMS patients with *COL13A1* mutations. If validated in further cases, this will help avoiding the immense costs of 3,4-diaminopyridine prescriptions which have become exorbitant since this medication has been licensed by a pharmaceutical company [8].

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Compliance with ethical standards

Conflicts of interest The authors declare no conflict of interest.

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