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Scientific Correspondence

Plectin-related scapuloperoneal myopathy with treatment-responsive myasthenic syndrome

The human plectin gene (*PLEC*) on chromosome 8q24 codes for a 4,684 amino acids large protein whose isoforms [1] are key for the functional and structural organization of filamentous cytoskeletal networks, thereby contributing to fundamental biomechanical properties of mechanical stress-bearing tissues [2]. Plectinopathies thus far comprise five autosomal-recessive entities, including epidermolysis bullosa simplex with muscular dystrophy (EBS-MD), EBS-MD with myasthenic syndrome (EBS-MD-MyS), limb girdle muscular dystrophy type 2Q, EBS with pyloric atresia, skin-only EBS and the autosomal-dominant variant EBS-Ogna [2,3]. Herein, we report the clinical, genetic, pathological and biochemical findings in a Caucasian patient with late-onset scapuloperoneal myopathy with ephedrine-responsive myasthenic syndrome caused by two novel compound heterozygous *PLEC* mutations. Informed consent in writing was obtained from the patient.

A 42-year-old Caucasian male presented with progressive symmetrical scapular and peroneal weakness and atrophy starting at age 25. He first experienced episodes of concurrent fatigue, fluctuating palpebral ptosis and diplopia, and dyspnoea in his early thirties. His past medical history included congenital hypotonia, delayed motor milestones and transient skin blistering limited to his early infancy. His motor performance was similar to his peers throughout childhood and adolescence except for mild endurance deficits. Family history was unremarkable. Neurological examination at the age of 36 showed external ophthalmoparesis, bilateral fluctuating palpebral ptosis, facial weakness, arched palate, and symmetrical and marked weakness and atrophy of the shoulder girdle muscles with scapular winging (**Figure 1A,B**) along with bilateral severe foot dorsiflexion weakness. He also presented severe weakness of his forearm and knee flexor muscles, moderate weakness of his hip flexors and extensors muscles as well as a rigid spine with prominent lumbar lordosis. His muscle weakness progressed, becoming a wheelchair user in his late thirties. At this point, Southern blot-based genetic testing for type 1 facioscapulohumeral muscular dystrophy (FSHD) was performed, which yielded normal results for D4Z4 microsatellite repeats. Creatine kinase levels were repeatedly elevated (10x above normal upper limit). A muscle MRI when aged 40 revealed pronounced fatty replacements of shoulder girdle muscles, the posterior compartment of thighs and the anterior compartment of his lower legs (**Figure 1C**). Electromyography revealed a myopathic pattern in conjunction with fibrillation potentials, positive sharp waves and complex repetitive discharges. Repetitive stimulation studies of the radial nerve at 3 Hz recorded a 20% decremental response. While oral pyridostigmine treatment alone (3 × 60 mg per day) or in combination with 3,4-DAP (3 × 15 mg per day, pyridostigmine reduced to 3 × 30 mg) showed no or only a slight benefit, a sole treatment with the sympathomimetic amine ephedrine (2 × 50 mg per day) led to a significant improvement of ptosis, diplopia and fatigue, and to a better score on the Myasthenia Gravis Composite Scale with a change from 15 to 7, especially on ocular-related items. While the further increase in the ephedrine dose (up to 2 × 100 mg per day) resulted in a complete remission of his myasthenic symptoms, this improvement was accompanied by typical sympathomimetic side effects comprising tachycardia and high blood pressure. Subsequently, the dose of ephedrine was reduced again to 2 × 50 mg per day and both pyridostigmine (3 × 30 mg per day) and 3,4-DAP (3 × 15 mg per day) were added, which led to a good control of his myasthenic symptoms. Morphological analysis of a diagnostic muscle biopsy taken from his right deltoid muscle showed a myopathic pattern with a slight increase in endo- and perimysial connective tissue, rounding of muscle fibres with increased fibre-size variability, and internalization of myonuclei (**Figure 1D, E**). The latter occasionally displayed clustering of atrophic and non-atrophic fibres. While neither degenerating and regenerating fibres nor rimmed vacuoles could be detected, multiple COX-negative fibres and fibres with rubbed-out lesions were present in combined COX/SDH stains (**Figure 1F**). In comparison to normal control tissue, plectin and desmin double-immunofluorescence analysis depicted a highly pathological picture with a

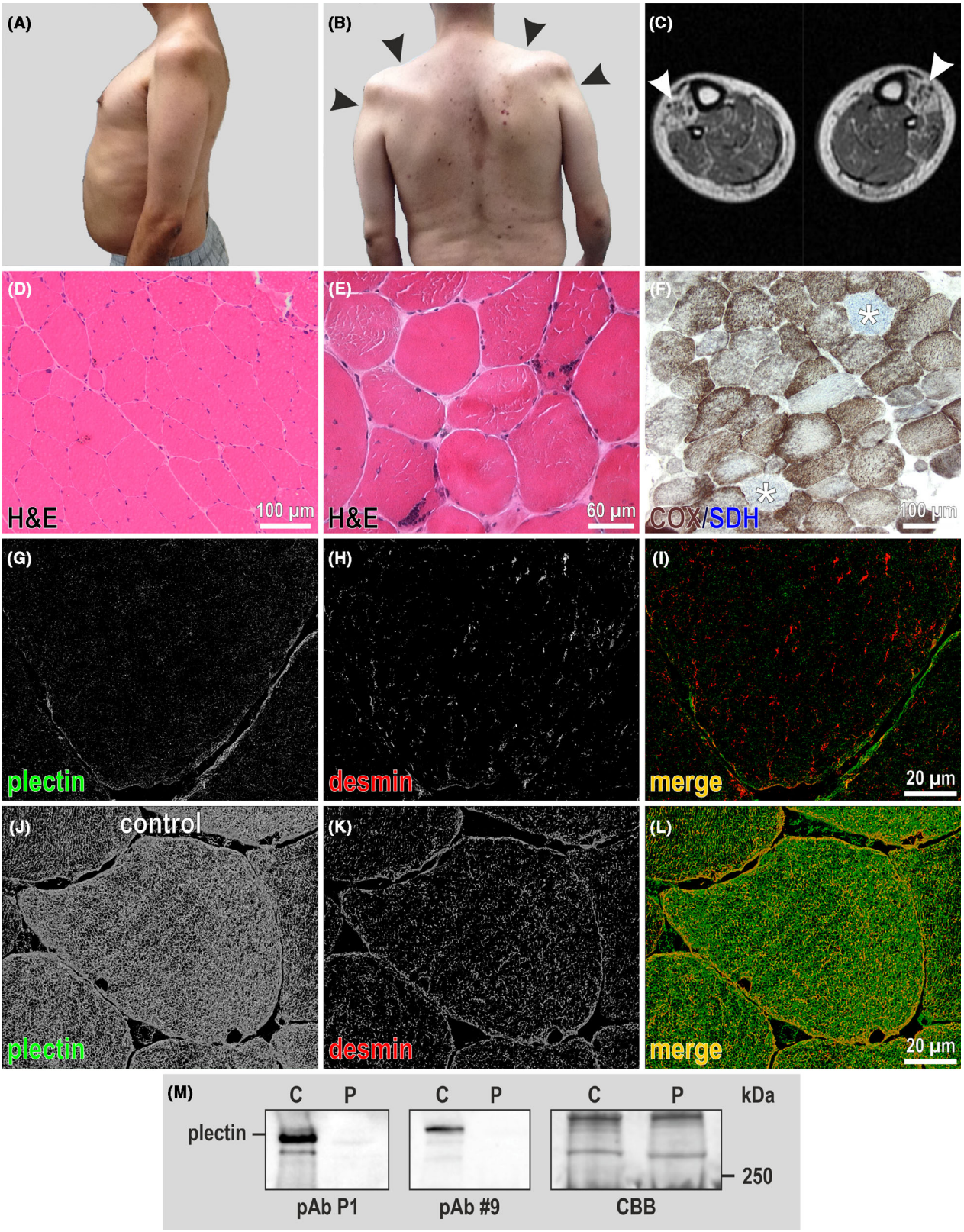

Figure 1. Clinical, radiological, myopathological and biochemical findings in a patient with EBS-MD-MyS. Note the marked atrophy of shoulder girdle muscles (**A**,**B**, arrowheads) and the marked fatty replacement of both peroneal muscle groups in the MRI picture of the lower legs (**C**, arrowheads). The diagnostic muscle biopsy showed a myopathic pattern (**D**,**E**) in conjunction with multiple COX-negative fibres (**F**, asterisks). Moreover, plectin and desmin immunostaining revealed markedly reduced signal intensities (**G**,**H**,**I**; normal control **J**,**K**,**L**). Immunoblots of total protein extracts from a normal (C) and the diseased (P) muscle using two different antibodies (P1, guinea pig polyclonal, C-terminal repeat domain 6 epitope, [11]; #9, rabbit polyclonal, N-terminal exons 9–12 encoded epitope [12]) directed against plectin demonstrated a virtually absent plectin signal in the patient (**M**). Note that the full-length plectin signals appear at different positions as sections of independent, immunoblotted SDS-PAGE gels are shown; (CBB) Coomassie Brilliant Blue stained SDS-PAGE gel for loading control.

complete absence of sarcoplasmic plectin staining and a coarsened and attenuated desmin signal pattern in all muscle fibres (**Figure 1G–L**). Traces of plectin-positive signals occasionally were detected at the level of the sarcolemma in a subset of muscle fibres (**Figure 1G**). Immunoblot analysis of total protein extracts using antibodies directed against the N- and C-termini of plectin further confirmed the nearly complete abrogation of plectin protein expression in his skeletal muscle tissue (**Figure** 1M). Sanger sequencing of the coding exons and the conserved splice sites of the *PLEC* gene resulted in the identification of two novel compound heterozygous variants. One variant consisted of a maternally inherited deletion of one nucleotide (NM_000445.3: c.11737delC) in exon 32 leading to a frameshift and a premature transcriptional termination 30 codons after the mutation (p.Arg3913Valfs*30), thus potentially yielding a protein of only 3,943 amino acids. The other variant was a heterozygous paternally inherited mutation affecting a highly conserved splice acceptor site (NM_000445.3: c.2539-2A > G). The latter mutation was predicted to lead to skipping of exon 21 resulting in frameshift and consecutive premature transcriptional termination with a resulting truncated protein of only 929 amino acids. Both truncated proteins were absent in immunoblots indicating they were not synthesized or were unstable (**Figure 1M** and data not shown). Thus, following ACMG criteria both variants were classified as novel, loss-of-function, disease-causing mutations. Using a custom-designed next-generation sequencing panel that included all known genes associated with myopathies and congenital myasthenic syndromes [4], we did not identify any pathogenic variant that segregated with the disease except for mutations in the *PLEC* gene. Thus, a genetic 'double-trouble' problem could be excluded in this patient.

Myopathies presenting with scapuloperoneal phenotype are caused by a wide variety of mutated genes involved in structural, enzymatic, transcriptional and homeostatic functions. This is the first report on an individual with adult-onset scapuloperoneal myopathy caused by two novel compound heterozygous *PLEC* mutations (c.11737delC in exon 32; c.2539-2A > G in exon 21) which affect the expression of all four skeletal muscle-specific plectin isoforms, subsequently causing the pathognomonic desmin and mitochondrial pathology in skeletal muscle tissue [5,6]. EBS-MD-MyS has only been reported in four patients so far [7–10]. Destruction and remodelling of junctional folds of neuromuscular endplates were reported in one patient [8], an observation also made in plectin knock-out mice [11]. Notably, our patient presented with transient skin blistering limited to his early infancy despite an almost total absence of plectin. A possible explanation is that minimal expression of the 3,943-amino acids protein, not detectable by Western blotting, could lead to its binding to the hemidesmosmal protein integrin α6β4, which, in turn, would bind to BPAG1e and BPAG2 (collagen XVII), allowing the formation of a partially functional hemidesmosome [12,13]. Hemidesmosome formation, even at limited levels, might then be responsible for the lack of an active skin phenotype. To date, no specific treatment is available for plectinopathies. In this context, it is noteworthy that a medication with ephedrine in combination with pyridostigmine and 3,4-diaminopyridine led to a marked improvement of myasthenic symptoms. It may thus serve as a symptomatic treatment to alleviate myasthenic symptoms in EBS-MD-MyS.

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Authors' contributions

HAE, NM and JJV performed and supervised the clinical, electrophysiological, and muscle biopsy and MRI analyses. DS, LK, MS, MT, CSC, MJC, GW, HH and RS performed or supervised the immunofluorescence microscopy and immunoblot analyses. CT was in charge of the genetic analyses. GW and HH critically revised the plectin protein expression data. HAE, DS, MT, CSC, JJV and RS prepared the figures and wrote the manuscript.

Disclosure

None.

Ethical approval

This study was approved by the research ethics committee from the Hospital Universitari i Politècnic La Fe (ref 2016/0905).

Data availability statement

Anonymized data used and analysed for this report will be shared upon reasonable request.

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