



Adult onset distal and proximal myopathy with complete ophthalmoplegia associated with a novel de novo p.(Leu1877Pro) mutation in MYH2

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Key Words:	MYH2, myosinopathy, oculopharyngodistal myopathy, ophthalmoplegia

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4 **p.(Leu1877Pro) mutation in MYH2**
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CONFLICT OF INTEREST

The authors have no conflict of interest to declare

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ABSTRACT

A *MYH2* mutation p.(Glu706Lys) was originally described in a family with autosomal dominant inheritance, where the affected family members presented with multiple congenital contractures and ophthalmoplegia, progressing to a proximal myopathy in adulthood. Another patient with a dominant mutation p.(Leu1870Pro) was described, presenting as a congenital myopathy with ophthalmoplegia. Here we present a patient with symptoms beginning at age 16 years, of prominent distal but also proximal weakness, bulbar involvement and ophthalmoplegia. Initially clinically classified as oculopharyngodistal myopathy, the patient was found to carry a novel, *de novo MYH2* mutation c.5630T>C p.(Leu1877Pro). This expands the phenotype of dominant *MYH2* myopathies with the clinical phenotype overlapping the oculopharyngodistal myopathy spectrum.

Key words: *MYH2*, myosinopathy, oculopharyngodistal myopathy, ophthalmoplegia.

INTRODUCTION

Cardiac and skeletal muscle myosins are hexameric proteins formed of two myosin heavy chains (MyHC) and two pairs of essential and regulatory myosin light chains¹. There are three main isoforms of MyHC in adult skeletal muscle. Slow/ β cardiac MyHC I, encoded by *MYH7* is expressed in slow, Type I fibres. MyHC IIa, encoded by *MYH2*, is expressed in fast, Type 2A fibres. MyHC IIx, encoded by *MYH1*, is expressed in Type 2B fibres¹. Mutations in *MYH2* have been associated with skeletal muscle disease¹, while mutations in *MYH7* have been associated with both cardiac and skeletal muscle disease^{1, 2}. Mutations in the genes encoding embryonic and neonatal myosin (*MYH3* and *MYH8*) have been associated with distal arthrogryposes¹.

MYH2 mutations cause dominant and recessive disease. Dominant mutations were first described in a family with congenital joint contractures, which largely resolve with age, and external ophthalmoplegia. Some patients later develop mild to moderate, predominantly proximal limb weakness^{3, 4}. Rimmed vacuoles, intranuclear and cytoplasmic inclusions in the muscle biopsies initially led to the entity being labelled as inclusion body myopathy 3 (IBM3)³. Recently, a patient was described with severe bulbar and respiratory involvement from birth, external ophthalmoplegia and a “waddling-steppage gait”, who had a *de novo* heterozygous c.5609T>C (reported as c.5737T>C), p.(Leu1870Pro) mutation of *MYH2*⁵. Recessive patients with homozygous or compound heterozygous mutations have severe external ophthalmoplegia and mild limb weakness.^{6, 7, 8} Thus, external ophthalmoplegia is a constant clinical hallmark of *MYH2* myopathies. Two other patients with only myalgia or mild proximal weakness had heterozygous variants in *MYH2*⁹, though these variants, from their frequency in public databases, are likely polymorphisms.

MYH7 mutations are associated with three major allelic diseases: cardiomyopathy, myosin storage myopathy and Laing distal myopathy¹. Cardiomyopathy most frequently results from mutations involving the MyHC I globular head domain¹, whereas myosin storage myopathy, manifesting predominantly as a scapuloperoneal syndrome, results from mutations in the distal tail of MyHC I^{1, 2}. Laing distal myopathy results most commonly from mutations in the mid region of the tail, although mutations in the distal tail and in the head domain have also been described².

The genetic basis of oculopharyngodistal myopathy (OMIM #164310) (OPDM) is unknown, but it shows both autosomal dominant and recessive inheritance. The main features are ophthalmoplegia, ptosis, bulbar dysfunction and distal limb weakness with onset spanning from 7 to 50 years.^{10, 11} Muscle biopsy features include rimmed and autophagic vacuoles¹⁰ and 16-18 nm cytoplasmic filaments in patients with recessive disease.¹²

We report a patient with ophthalmoplegia, dysphagia and limb weakness, with significant involvement of distal muscles from onset, initially diagnosed as OPDM, and harbouring a mutation in *MYH2*.

PATIENT AND METHODS

Case report

An Australian male of Italian background, born to non-consanguineous parents, presented at the age of 20 years with a four year history of difficulty climbing steps and a tendency to invert his feet. He had three healthy siblings, and no affected relatives. His motor milestones were normal. Examination demonstrated generalized atrophy of muscles in upper limbs, asymmetric scapular winging and facial weakness. He had a positive Gower's sign and foot drop. At 23 he developed weakness of the intrinsic hand muscles. By 25 he had complete ophthalmoplegia, except for partial preservation of downgaze. Mild bilateral ptosis developed by age 32, as well as reduced palatal movements and a hypophonic voice. Pectoralis, deltoid, biceps, triceps, and wrist flexors and extensors were graded 4/5. The intrinsic muscles of the hand were globally graded as 3-/5. Flexion of hips and knees were estimated to be 3/5. Dorsiflexion and plantar flexion of the foot were 3/5 and inversion of the foot 3+/5. Deep tendon reflexes were absent, except for reduced ankle jerks.

He slowly deteriorated, developing dysphagia and respiratory involvement with a moderate restrictive ventilatory defect. His forced vital capacity was 57% predicted, necessitating intermittent CPAP. He had severe contractures of his ankles, but was able to walk with full leg calipers until age 50.

Electromyography performed at age 25 showed normal nerve conduction studies but a widespread myopathic pattern.

A muscle MRI performed at age 58 showed extensive fat replacement. The deltoids and anterior compartment of the legs were relatively spared. Medial heads of gastrocnemius were less affected than lateral heads (Fig. 1).

His serum creatine kinase (CK) ranged between 153 and 806. ECG and echocardiogram performed at age 44 were both normal. Both parents had normal CK levels.

Histology

Five muscle biopsies were performed at age 20 (right biceps and right vastus lateralis), 32 (right vastus lateralis) and 46 (left vastus lateralis and left deltoid).

The biopsies were stained with haematoxylin and eosin, ATPase pH 9.4, 4.6 and 4.3, metabolic stains and a picro-Mallory's stain. Slow (Milipore, clone MAB1628) and fast myosin (Sigma, clone MY-32) immunohistochemistry (IHC) was performed on paraffin embedded tissue. IHC was performed for dystrophin (Rod, C-terminal and N-terminal domains), α , β , γ , and δ sarcoglycans, α and β dystroglycan, merosin, dysferlin, caveolin3 and spectrin.

Genetic analysis

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3 Genomic DNA was extracted from blood using standard procedures. Mutations in various genes were excluded as being
4 causative, including: facioscapulohumeral muscular dystrophy D4Z4 repeat contractions, large scale deletions of
5 mitochondrial DNA, *ANO5*, calpain 3, collagens 6A1, 6A2, and 6A3 and exons 7, 8, 13, and 16-18 of *POLG* and deletion of
6 exon 7 of *SMN1*.
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10 Targeted capture and next generation sequencing (NGS) of 277 known and candidate neuromuscular disease genes
11 (manuscript in preparation) was then performed. Bioinformatic analysis of the sequencing data used the previously
12 published in-house pipeline¹⁴. Confirming the presence of the *MYH2* variant and screening of relatives was done by Sanger
13 sequencing.
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18 RNA was extracted from the patient's vastus lateralis biopsy taken at 20 years of age and controls using RNeasy Fibrous
19 Tissue Mini Kit (Qiagen). Synthesis of first strand cDNA was performed using SuperScript® III First-Strand Synthesis
20 (Invitrogen). To analyse the relative expression of MYH isoform transcripts, we performed a multiplex PCR and fragment
21 analysis as described.¹³ MYH2 cDNA exons 36 to 40 were sequenced using primers specific for *MYH2* (available on request).
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26 RESULTS

27 Histology

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29 Common features in all the biopsies were increased variability in fibre size, atrophic and hypertrophic fibres ranging from
30 20 to 250 µm in diameter, internal nuclei (up to 15%) and endomysial fibrosis. Dystrophic features were present, being
31 more prominent later in the disease. Deltoid was relatively spared compared to vastus lateralis (Fig. 2).
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38 Oxidative enzyme stains demonstrated abundant lobulated fibres, and core-like structures. In some areas, all fibres were
39 lobulated except for scattered hypertrophic fibres which showed central cores (Fig. 2E and 2G). Electron microscopy
40 confirmed these findings (Fig. 1M-P). No rimmed vacuoles were seen. All fibres were Type 1, except for a few scattered
41 small Type 2 fibres. This fibre type distribution was found in deltoid (Fig. 2I-L) and vastus lateralis (not shown).
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45 Immunohistochemical staining showed normal labelling of all other proteins examined (not shown).
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48 Genetic work-up

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50 NGS for the patient's DNA covered 92.4% of targets to >20-fold, with 247-fold average coverage. After filtering variants in
51 the EVS (<http://evs.gs.washington.edu/EVS/>) or 1000 genomes databases (www.1000genomes.org) with frequency >1%, a
52 number of heterozygous variants in genes associated with autosomal recessive diseases, but no homozygous or compound
53 heterozygous variants, were identified. Two variants were identified in genes associated with dominant diseases.
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3 1) A heterozygous variant in Filamin C (FLNC) (NM_001458) c.C5578T(p.R1860C) present in EVS with a frequency of 0.46%,
4 thus estimated unlikely to be pathogenic. However, since mutations in *FLNC* have been associated with distal myopathies,
5 relatives were checked for the variation showing that the unaffected mother carried it.
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9 2) A heterozygous change in exon 39 of *MYH2* (NM_017534.5): c.5630T>C p.(Leu1877Pro). This variant was not present in
10 the EVS or 1000 genomes databases. The putative amino-acid change involved a highly conserved amino acid (Fig. 3B), and
11 would insert a proline residue in the coiled-coil tail of MyHC IIa. This was predicted to be deleterious by all in-silico
12 predictors used (Mutation Taster, PolyPhen2 and SIFT). The variation was confirmed in the proband by Sanger sequencing,
13 but was not present in peripheral blood DNA of either of the parents, or the 2 unaffected siblings for whom DNA samples
14 were available. Sample identity was confirmed, suggesting the proband had a *de novo* mutation, or one parent had gonadal
15 mosaicism for the mutation.
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18 To exclude the possibility of a second mutation causing a null allele, we sequenced exons 36 to 40 on cDNA showing that
19 both the wild type and the mutant allele were expressed, although the mutant seemed present in a lower amount, as
20 inferred from the smaller peak corresponding to the mutant allele, seen in the chromatogram (Fig. 3A).
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23 To further investigate the effect of the mutation, we quantified the MYH isoform transcripts in the patient muscle. This
24 showed almost complete absence of both *MYH1* and *MYH2* transcripts (Fig 3C), as expected from the very few Type II
25 fibres seen in the biopsies.
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28 DISCUSSION

29 We describe only the third dominant mutation to be identified in *MYH2*. The MyHC IIa p.(Leu1877Pro) mutation is only
30 seven amino acids C-terminal of the p.(Leu1870Pro) mutation identified in the patient of D'Amico et al⁵. The two variant
31 amino acids are both at the "d" position of the coiled-coil heptad repeat¹. Similar dominant missense mutations to proline
32 in the coiled-coil tail domain of MyHC I are a principal cause of both Laing distal and myosin storage myopathies² and have
33 been identified at all seven positions of the heptad repeat, indicating insertion of a proline at any repeat position is
34 deleterious².
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37 The muscle pathology in our patient and that of D'Amico et al⁵, with Type 1 uniformity apart from a few scattered small
38 Type 2 fibres, is almost identical. This has also been described in patients with recessive mutations^{7, 8}. How dominant
39 missense mutations to proline in the MyHC IIa tail lead to loss of both Type 2a and 2b fibres is currently unknown. The
40 p.(Leu1877Pro) variant involves the first amino acid of the "assembly competence domain" of MyHC IIa (Fig. 3) which is a
41 29-residue motif essential for self-assembly of myosin molecules¹⁵. The mutations in our patient and that of D'Amico et al⁵
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3 may therefore sufficiently disrupt thick filament assembly to cause loss of Type 2a fibers. The up to 100% abundance of
4 lobulated fibres has not been described previously in patients with *MYH2* mutations. Lobulated fibres are regarded as non-
5 specific, of uncertain significance and their pathophysiology is unknown but it is unusual to find them in such high
6 proportion¹⁶.
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10 In our patient, changes were more prominent in vastus lateralis than in deltoid as seen clinically, pathologically and
11 radiologically. However, the anterior compartments of lower legs were severely affected clinically, but the MRI showed
12 relative sparing. This also seems to be the case in the patient of D'Amico et al⁵, where a steppage gait is described at age
13 12 but the MRI shows sparing of tibialis anterior. A common radiologic pattern can thus be established with D'Amico et al's
14 patient, though in our patient the MRI was performed much later in the disease progress. The pathobiological basis of the
15 preferential susceptibility or sparing of muscles in MYH2 myopathy, as in other genetic muscle diseases, is unknown, but
16 may involve the relative expression of other genes or protein isoforms in different muscles.
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20 Our patient was initially diagnosed with OPDM since the distribution of weakness, including significant distal weakness at
21 onset, ophthalmoplegia and dysphagia, suggested this diagnosis. Significant distal limb weakness is not generally a feature
22 in patients with MyHC IIa myopathies⁴⁻⁸, but may be inferred for the patient described by D'Amico et al from the
23 "waddling-steppage gait" clinical description⁵. Equally, some OPDM patients may have only proximal limb weakness or no
24 limb weakness at all¹⁰. Rimmed vacuoles and tubulo-filamentous inclusions have been reported in both diseases^{3, 12}.
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26 Dystrophic features may occur in severely affected muscles of MyHC IIa myopathy but have not been reported in OPDM.
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28 Nevertheless there is undoubtedly clinical and pathological overlap between MyHC IIa myopathy and OPDM. *MYH2* should
29 be considered a candidate gene in patients with distal weakness and ophthalmoplegia.
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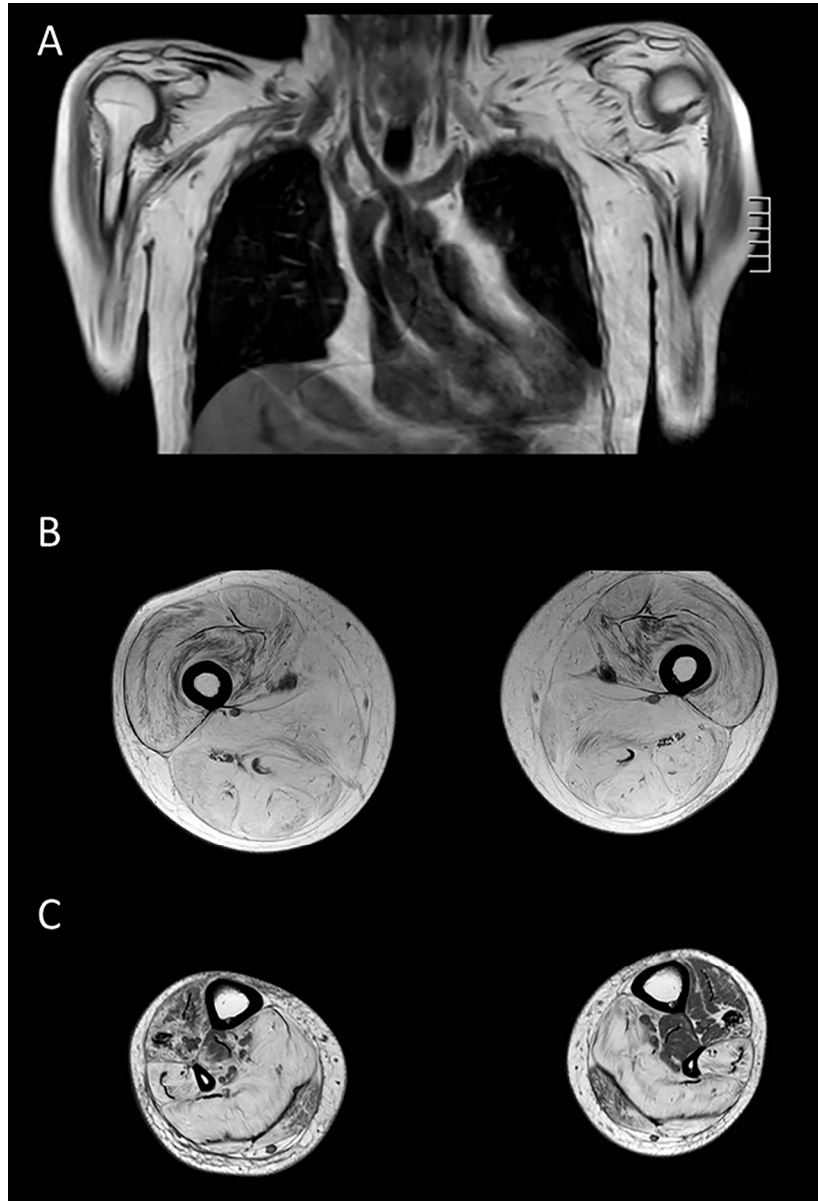


Figure 1: Muscle MRI

A: Coronal upper body T1-weighted sequence: Relative preservation of deltoid bilaterally. B: Axial thighs T1-weighted sequence: Severe widespread fat replacement involving muscles in anterior and posterior compartments. C: Axial calf T1-weighted sequence: severe fat replacement of muscles in posterior compartment, only remaining some muscle tissue in the medial head of gastrocnemius. The involvement is less severe in the anterior compartment with relative sparing of tibialis anterior and tibialis posterior.

104x152mm (300 x 300 DPI)

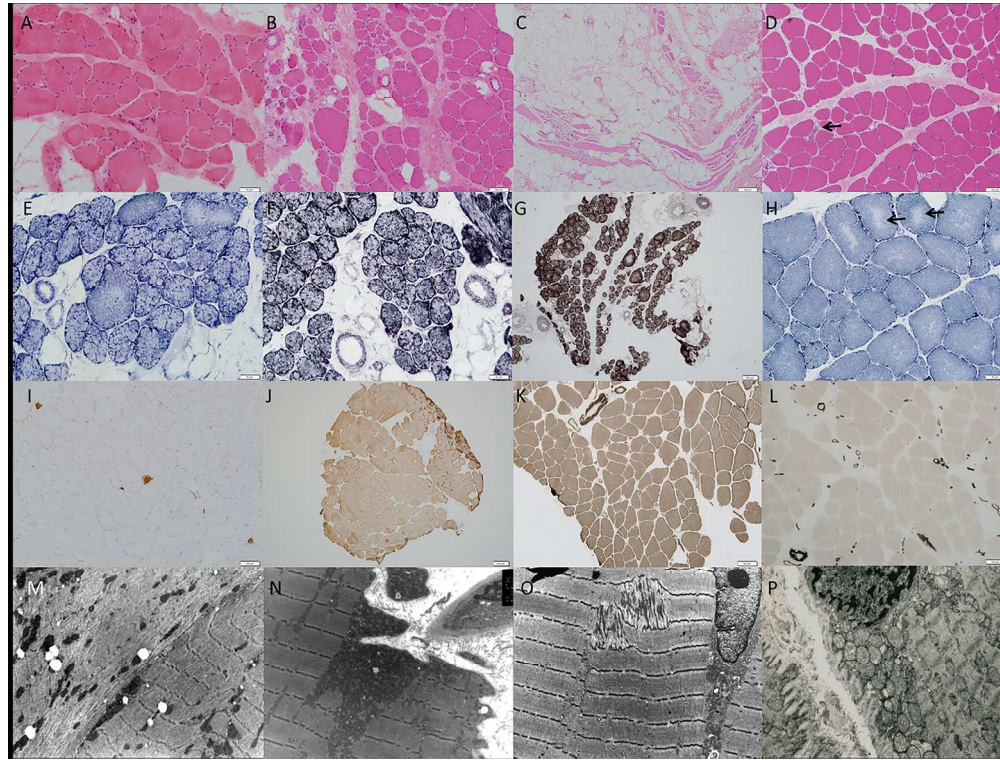


Figure 2: Muscle pathology

A to D: Haematoxylin and Eosin. E and H: NADH, F: SDH, G: COX, I: Fast myosin. J: slow myosin. K: ATPase 4.3. L: ATPase 9.5. M to P: Electron microscopy

A: Vastus lateralis biopsy taken at 20 years of age; increased endomysial and perimysial connective tissue. B: Vastus lateralis biopsy taken at 32 years of age; more advanced dystrophic pattern, showing increased endomysial connective tissue, high variability in fibre size with some atrophic and hypertrophic fibres. C: Vastus lateralis biopsy taken at 46 years old; end stage muscle. Widespread connective and fat tissue replacement with a few muscle fibres remaining. D: Deltoid biopsy taken at 46 years of age; dystrophic pattern with variability in fibre size, increased endomysial connective tissue and regenerating fibres (arrow). E, F and G: vastus lateralis biopsy taken at age 32; the majority of the fibres are lobulated fibres. H: Deltoid biopsy taken at age 46; core-like areas (arrows) in some hypertrophic fibres. I: Deltoid biopsy taken at age 46; few scattered atrophic fibres expressing fast myosin. J: Deltoid biopsy taken at age 46; all fibres expressing slow myosin. K: Deltoid biopsy taken at age 46; all fibres are type I. L: Deltoid biopsy taken at age 46; no type 2 fibres are seen. M: myofibril disorganization showing a core. N: triangular shaped subsarcolemmal mitochondrial accumulations in keeping with a lobulated fibre. O: Areas of Z band streaming. P: subsarcolemmal accumulations of structurally normal mitochondria.

279x210mm (300 x 300 DPI)

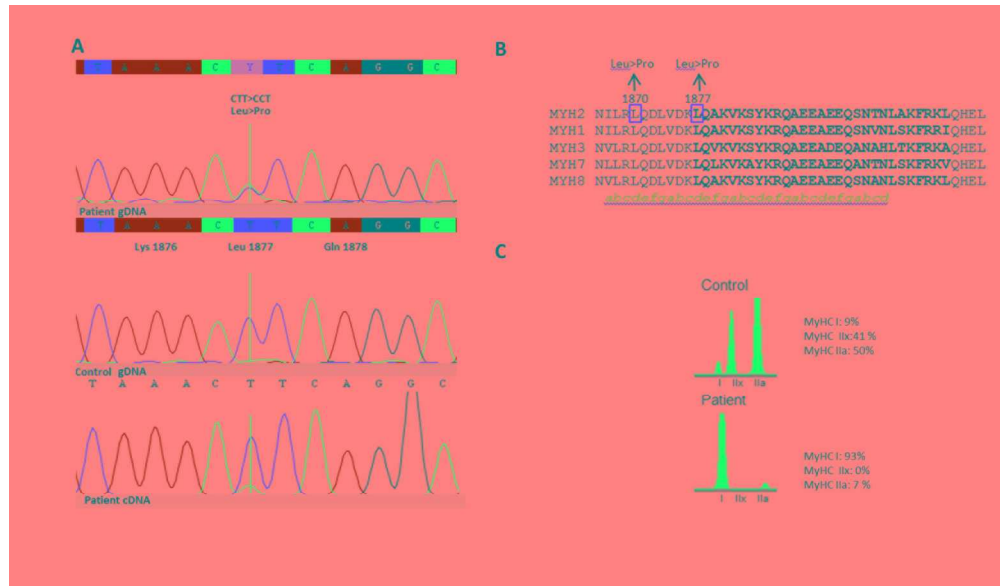


Figure 3: Chromatogram and protein isoforms alignment

A: Chromatograms from Sanger sequencing of exon 39 of MYH2 in genomic DNA (gDNA) of the patient and a normal control and cDNA of the patient showing a heterozygous T>C change involving a leucine at residue 1877 in the patient's gDNA and cDNA.

B: Alignment of amino acids sequence of a region in the tail of myosins expressed in human skeletal muscle indicating the assembly competence domain (bold) and correspondence with positions at the heptad repeat. The positions of the mutation in our patient (1877) and in the patient reported by D'Amico et al (1870) are highlighted (red boxes). Both mutations produce a change from leucine to proline at a "d" position in the heptad repeat. The mutation in our patient involves the first residue of the ACD, and the mutation described by D'Amico et al is seven residues upstream. Both amino acids are conserved among the skeletal muscle myosins.

C: Expression of MYH isoform transcripts. The quantitative analysis of the MYH isoforms transcripts based on the simultaneous PCR cDNA amplification and fragment analysis in the patient and an age-matched control showed severe reduction of MYH2 (MyHC IIa) and MYH1 (MyHC IIx) isoforms in the patient.

149x87mm (300 x 300 DPI)