A novel MYH7 founder mutation causing Laing distal myopathy in Southern Spain

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Highlights

- We report the novel variant p.R1560P in *MYH7* gene as a founder mutation in southern Spain
- Most patients display the classic Laing distal myopathy phenotype
- Interestingly, neck flexor weakness is revealed as early sign in some cases
- MRI shows early involvement of the sartorius muscle, even in asymptomatic carriers

ACCEPTED MANUSCRIPT

TITLE: A novel *MYH7* founder mutation causing Laing distal myopathy in Southern Spain

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ABSTRACT

MYH7 gene mutations are associated with wide clinical and genetic heterogeneity. We report a novel founder mutation in *MYH7* in Southern Spain (Andalucía). We studied two index patients and 24 family members from two apparently independent families by physical examination, serum creatine-kinase, muscle MRI, sequencing studies and genetic linkage analysis. Sixteen individuals were heterozygous for a (p.R1560P) variant in the *MYH7* gene. Haplotype was consistent with a common ancestor for the two families. The patients displayed the classic Laing distal myopathy phenotype, with hanging first toe as the initial presentation, even in mildly affected patients who declared themselves asymptomatic, although neck flexor weakness was revealed as an early sign in some cases. MRI showed that the sartorius was the first muscle involved, even in two out of three asymptomatic carriers. Our findings support the novel variant p.R1560P in *MYH7* as a founder mutation in Andalucía. The early involvement of the sartorius muscle in MRI may be useful as an indicator of affection status.

Keywords: *MYH7*; founder mutation; distal myopathy; muscle MRI; genetic linkage analysis

INTRODUCTION

Laing distal myopathy is an autosomal dominant disease, commonly characterized by slowly progressive distal weakness, involving the anterior compartment of the legs, thus causing the typical “hanging big toe” sign [1]. Nevertheless, highly variable clinical and histological phenotypes have been widely reported in Laing distal myopathy [2-4], and the

same mutation in the *MYH7* gene can cause a different clinical picture regarding age of onset, weakness pattern or severity and variable pathologic profiles, even within the same family [3].

Concerning muscle MRI in the Laing distal myopathy, the tibialis anterior is the earliest and more severely affected muscle in all the reported cases. To date, several case reports [3, 5, 6] and one study with detailed analysis on radiological pattern in a cohort of Laing distal myopathy patients [7] have been published, reporting quite variable and not always consistent conclusions regarding the muscle imaging findings other than the tibialis anterior early involvement.

The largest series of distal myopathy patients with mutations in *MYH7* reported a high rate of *de novo* mutations. This high frequency of sporadic mutations suggest that there should be a wide geographical distribution [7-9]. Exceptionally, a founder mutation in the *MYH7* gene, p.K1729del has been found to cause the majority of Laing distal myopathy cases in the Safor region, Spain [10]. Most mutations are associated with the classic phenotype of Laing distal myopathy. However, in the series reported by Fiorillo et al [7] some atypical clinical features were reported which delayed the diagnosis.

Lamont et al reported eight out of 15 families with missense mutations introducing a proline in *MYH7* associated with distal myopathy [9]. These mutations involve amino-acid residues at all positions of the heptad repeat of the protein which would induce significant modifications in the protein structure, which suggests that proline substitutions at any of these positions has a pathogenic effect [11, 12].

Here, we report a novel mutation in the *MYH7* gene with a founder effect in Andalucía (South of Spain). The description of founder mutations and the analysis of a potential genotype-phenotype correlation are important from a diagnostic point of view, especially when variable phenotypical expression is associated with the disease.

METHODS

Patients and study protocol

We initially identified two apparently unrelated patients with a distal anterior myopathy, both from Andalucía (Fig. 1). Patient II.6/Family A reported several affected relatives including his father and at least four siblings. Patient II.3/Family B was a sporadic case. Affected and unaffected relatives from both families were contacted and offered the possibility of participating in the study. The participants were examined using a standardized protocol which included interview, physical exam, muscle MRI, serum CK and blood sample to obtain DNA. The first step of the clinical protocol was the interview of the recruited family members to collect the clinical data and classify them as symptomatic or asymptomatic, followed by a complete general and neurological examination, including manual muscle testing, and the rest of the tests. This study received approval from the HUVR local Ethics Committee and all participants provided informed consent.

Muscle biopsy

Muscle samples were obtained by open biopsy. We processed the muscle following the standard procedures. Routine stains included hematoxylin & eosin (HE), modified Gomori trichrome, Oil Red O, periodic acid-Schiff (PAS), nicotinamide adenosine dinucleotide dehydrogenase (NADH) nicotinamide adenine dinucleotide (NADH), succinate dehydrogenase (SDH), cytochrome oxidase (COX), and combined COX/SDH, and myofibrillar adenosine triphosphatase (ATPase) after preincubation at pH 9.4, 4.5 and 4.3. Immunohistochemistry for fast and slow myosin was performed in one case.

Muscle MRI

Muscle MRI was performed at the level of the thighs and lower legs. We used a 1.5 Tesla scanner (MRI Intera, Philips Medical Systems). Axial MRI sections were obtained including a T1-weighted spin-echo sequence (TR 600-700 ms, TE 30 ms) and a short-time inversion recovery (STIR) sequence (TR 2500-3500 ms, TE 60 ms, TI 150 ms), in 10 mm slices. Fatty degeneration of muscles was identified according to the increased signal in T1 sequences and quantified according to a subjective scale [13], and STIR sequences were used to evaluate the presence of edema.

MYH7 genetic study

Genomic DNA was extracted as per standard protocols from peripheral blood (Chemagic DNA Blood 5K Kit, Perkin Elmer) or saliva samples (Oragene DNA Self-Collection Kit, DNA Genotek) from patients and the rest of family members. In the index patient II.6/Family A, the coding regions of *MYH7* gene were Sanger-sequenced at the Neuromuscular Unit, Hospital U. Virgen del Rocío, Sevilla (NMU-HUVR). DNA from index patient II.3/Family B was sent to the Lotterywest State Biomedical Facility Genomics (Perth, Australia) for neurogenetic sub-exomic supercapture and next generation sequencing (Proton™, Life Technologies) of 336 genes, which included 254 disease genes listed in the Neuromuscular Disorders Gene Table (www.musclegenetable.fr). DNA samples from all recruited relatives were PCR-amplified using specific primers (5'-CCATGATTAGTGAGCAGGCC-3', 5'-TCAGAGGTCCCTGCAGTAAC-3') and directly sequenced using BigDye Terminator v3.1 Cycle Sequencing kit (Thermo Fisher Scientific).

Haplotype analysis

Microsatellite and SNP markers covering the *MYH7* gene were selected based on their genomic location and heterozygosity (UCSC Genome Browser, Human GRCh37/hg19; <http://genome.ucsc.edu/>) [14]. They spanned a genomic region of 2,92 Mb. Microsatellite markers were amplified by PCR using fluorescently-labelled primers and resolved in an ABI 3500 Genetic Analyzer (Applied Biosystems). SNP *loci* were PCR-amplified and genotypes were determined by visual inspection of the sequence chromatograms. Haplotypes were built assuming the minimum number of recombinations.

RESULTS

Clinical phenotype

We performed the interview and physical examination in 23 individuals from the two families, nine women and 14 men. The age at the time of the examination ranged from eight to 79 years. The symptoms appeared between four and 30 years of age. Most carriers recalled abnormal gait and falls during infancy but did not complain of weakness until adulthood. Twelve carriers of the p.R1560P mutation (age range 8-79 years) showed a pathological physical exam. Three of them (age range 21-42 years) declared themselves to be asymptomatic and weakness restricted to big toe extension was the only sign found in the exam (III.4/Family A, II.5/Family B, III.2/Family B). Two of them presented transient cervical weakness at eight years old but not later (III-8/Family A, III-15/Family A). Three out of the 16 carriers were asymptomatic with normal physical exam (age range 11-38 years). One carrier was not examined (I.2/Family B). No patient referred respiratory or cardiac symptoms, and none of them referred a familial history of sudden death,

arrhythmia or cardiomyopathy. Echocardiography and respiratory functional studies were performed in some affected patients and all the studies were normal. CK was in normal range in all the carriers. Clinical findings are summarized in Table 1 and Figure 2.

Muscle histology

Muscle biopsies from four patients (II.5/Family A, II.6/Family A, II.14/Family A, II.3/Family B), two tibialis anterior and two gastrocnemius muscles, were available for review. Myopathic pattern with increased variability in fiber size and internal nuclei were present in all biopsies. More severe changes with prominent atrophy and increased endomysial connective tissue were observed in both tibialis anterior muscles (Fig 3A and 3B). Small group of angulated fibers and nuclear clumps suggesting neurogenic features were seen in three of the four samples (Fig 3A and 3F). Oxidative enzyme stains (NADH, SDH and COX) revealed prominent internal sarcoplasmic disorganization with core, core with poor defined borders and minicore lesions in all biopsies (Fig 3C and 3G). We observed type I fiber predominance in three cases (Fig 3D) and scattered rimmed vacuoles in one case. No hypotrophy of type I fibers was observed.

Imaging study

Muscle MRI was performed in 11 patients (Family A: II.5, II.6, II.14, III.2, III.4, III.14, III.15; Family B: II.3, II.5, III.1, III.2). All the affected carriers with moderate or severe phenotype (mean age 54.1 ± 12.2 years old [range 39 to 79 years], disease duration 44.6 ± 17.2 years [range 9 to 66 years]) showed fatty degeneration of lower limb muscles. The tibialis anterior, gluteus major and sartorius muscles were involved in all of them, although with different degrees of degeneration. Degeneration was severe in the tibialis anterior and mild in the gluteus and sartorius muscles. The other affected muscles in the MRI were the gracilis (75%), the quadriceps (50%) and the gastrocnemius (25%). Two slightly affected patients (III.4/ Family A, II.5/Family B) and two asymptomatic carriers

(III.2/Family A, III.1/Family B) presented mild fatty infiltration only in the sartorius muscle. Two mildly affected patients (III.15/Family A, III.2/Family B) and one asymptomatic carrier (III.14/Family A) showed normal MRI. The sartorius muscle was the only one involved in 50% of slightly affected patients and in 66.6% of asymptomatic carriers. MRI findings are summarized in Table 2 and Figure 4.

MYH7 mutation screening

The novel heterozygous mutation c.4679G>C (NG_007884.1) p.R1560P in exon 34 of *MYH7* was identified in both index patients (Fig. 1). The variant is absent from databases of controls (<http://gnomad.broadinstitute.org/>; last accessed in November 2017) and is predicted to introduce a proline in the slow myosin tail. Next generation sequencing in the index patient II.3/B excluded pathogenic mutations in other genes related to neuromuscular disorders. The novel variant was subsequently analyzed by Sanger sequencing in all the recruited family members. In total, 26 subjects from the two families were recruited (17 from Family A and nine from Family B), comprising twelve clinically affected patients and 14 asymptomatic individuals. Segregation analysis showed that 16 individuals were heterozygous for variant p.R1560P in the *MYH7* gene, including twelve symptomatic individuals.

Haplotype analysis

Given the identification of the same novel mutation in two families from different cities in Andalucía, Southern Spain, we studied the possibility of them being related. Haplotype analysis revealed a shared genomic region of 306 Kb in all carriers of p.R1560P mutation, restricted to four SNP markers (Fig 5).

DISCUSSION

We report a novel founder mutation in *MYH7* causing Laing distal myopathy in Andalucía. The shared haplotype identified in our study spans a short genomic region (306 Kb) defined by SNP markers, however, the data are in accordance with the findings by Muelas et al. [10], that show a common SNP-defined genomic region of 41,2-71,2 Kb. In our Neuromuscular Unit at HUVR, which is a national reference unit, only patients with ancestors from Southern Spain carry the novel c.4679G>C (p.R1560P) mutation in *MYH7*, while in patients from other geographic regions of Spain the disease is due to different mutations, all of them already described. Thus, this mutation should be the first mutation to study in patients with this disease phenotype from Southern Spain. Proline residues are widely known to have a pathogenic effect on the assembly of the protein [15, 16]. Moreover, most mutations in *MYH7* associated with distal myopathy are located in the mid-rod region of *MYH7* protein encoded by exons 32 to 36 [17, 18]. All together, these findings support the pathogenic role of this novel mutation. The high rate of *de novo* mutations demonstrated in *MYH7* together with the long lifespan of patients with Laing distal myopathy could explain the high prevalence of cases, regardless of geographic or ethnic distribution [7, 9]. Hence, the identification of founder mutations in specific geographic areas facilitates the molecular diagnosis of patients.

A noticeable clinical and genetic heterogeneity is associated with the *MYH7*-related myopathies [3, 4, 9]. Until now, only one founder mutation in *MYH7* has been reported, and it was also associated with a wide range of clinical and morphologic features among the carriers [3, 10]. In this study, we identified the clinical variability previously reported in other series, finding patients severely or mildly affected at a similar age, in the same family. Four previously reported large series of patients showed that age at onset varied between 0 and 50 years [3, 7, 9, 19] and in our series the onset was between four and 30 years of age (none of the patients with congenital onset), and the asymptomatic ones

ranged from 11 to 38 years old. Fiorillo et al. described a series of 21 patients identifying 12 heterozygous mutations in *MYH7* affecting the C-terminal rod domain, and the patients with congenital or early onset presented predominant distal weakness, while patients with later onset presented prevalent proximal weakness [7]. In contrast, in our series with a novel mutation affecting the rod domain we found no differences in the distribution of the weakness related to the age of onset. In our cohort, we found three asymptomatic carriers (age ranged 11-38 years old) and five slightly affected patients (age ranged 8-42 years old). Tasca et al. reported one family carrying a novel mutation in the myosin tail, and the age of onset of all three affected members was not prior the fifth decade and shared mild weakness limited to long toe and foot extensors [4]. Feinstein-Linial et al., who reported two novel heterozygous proline substitutions located in exon 31 of *MYH7*, described also one asymptomatic carrier aged 40 [5]. This confirms that more benign forms than the classic phenotype exist, probably associated with better prognosis regarding the progression of the disease.

We uncovered interesting manifestations after a thorough anamnesis and clinical exam. The majority of carriers of the mutation reported abnormal gait with increasing tendency to stumble and fall in infancy or childhood, although most of them did not complain of motility or muscle problems until the third or fourth decade, a finding also described by Muelas et al [3]. A review of 34 patients with Laing distal myopathy by Lamont et al. revealed that initial weakness of great toe/ankle dorsiflexion and later development of neck flexor weakness were consistent findings [9]. Interestingly, two infantile carriers in our series presented neck flexor weakness only during childhood. Recently, one atypical case with dropped head at the age of four has been reported, but authors do not refer if it disappeared later and revealed also calf hypertrophy and cardiopathy at the age of eight [7]. Thus, we must be attentive to abnormal gait or cervical weakness during infancy

because it can be the first transient symptom or sign of the disease. Hanging big toe is the typical sign of this disease, it is frequently the first obvious sign [4], even we uncovered hanging big toe in three mildly affected patients who self-reported no symptoms, as already reported by Muelas et al. in two patients [3]. However, we observed no correlation between the severity of the toe extensor weakness and the severity of other manifestations. Thus, weakness of the big toe extension may represent the most consistent clinical sign in every stage of the disease, although neck flexor weakness and abnormal gait during infancy could represent the first manifestation in some cases. Calf hypertrophy and pes cavus are findings reported in other families [3, 4], and we found these symptoms only in family A, so they do not seem to be specific to the novel mutation c.4679G>C.

Regarding the muscle biopsy, our series also showed some of the variable morphological findings previously reported: hypotrophy of type I fibers, fiber type disproportion, areas devoid of oxidative activity forming cores/pseudocores, angulated fibers, nuclear clumps, and rimmed vacuoles [3, 9, 19]. However, which is the most prominent morphological feature varies between series, thus atrophic type I fiber was found as the most common abnormality in some previous series but it was not observed in our study, while cores/pseudocores were highly frequent in most of the series [3, 9] and ours but were not described by Lamont et al [19].

We have reviewed the MRI descriptions reported until now, demonstrating variability in radiological findings [3, 4, 6, 7]. As previously described, in our series the tibialis anterior muscle becomes the most severely degenerated muscle in early stages of the disease, and the gastrocnemius medialis muscle was simultaneously affected in one patient which is in accordance with recent reports showing involvement of the gastrocnemius medialis in about half of the patients [5-8]. However, some authors described that the gastrocnemius together with the gracilis and the sartorius muscles are usually preserved even in severely

affected patients [3, 20], and our data contradict this assertion. We observed that the involvement of the sartorius muscle is a very early radiological finding in our patients, even in presymptomatic stages. This is quite surprising, since the sartorius muscle is usually preserved until late stages in many other neuromuscular disorders, except for some myofibrillar myopathies where it seems to be a hallmark [21]. The involvement of the adductor magnus and the sartorius muscles has only been mentioned in few reports of Laing distal myopathy, with no further analysis regarding their evolution [5, 7]. We observed that the degree of involvement of the sartorius is always mild at early stages, showing no further progression of the degeneration during the course of the disease. Although this has not been described as a typical radiological sign in Laing distal myopathy, our results support that the mild involvement of the sartorius muscle is a common and early radiological sign in this myopathy, prior to the involvement of the tibialis anterior muscle, even in asymptomatic carriers, so it could be a useful sign for screening.

To conclude, our findings support the novel variant p.R1560P in *MYH7* as a founder mutation in Andalucía, and it should be considered the first mutation to study in patients from Southern Spain. These large pedigrees allowed us to better describe the progression and diagnostic clues in very early stages of the disease, such as neck flexor weakness, hanging big toe and the sartorius muscle degeneration in muscle MRI, which could be useful for detecting asymptomatic carriers of the *MYH7* mutation. All these findings are important to improve the diagnosis and the genetic counseling of Laing distal myopathy.

AUTHOR CONTRIBUTIONS

C.P. and P.C designed the study. E.T.C and A.M.M performed the haplotype analysis. E.S., C.P., P.C., L.V., M.C.S., E.A-G, G.G., A.G-G., and M-I.C. handled patients and collected biological samples. M.C.S. and N.L. performed next generation sequencing and analyzed the results. E.R. processed and studied muscle biopsy. C.P. and P.C analyzed the radiological findings. C.P., A.M.M. and A.M-V. supervised and provided critical discussion on the research. C.P. coordinated all the study. P.C wrote the initial manuscript. All authors contributed to the final version of the manuscript.

CONFLICT OF INTEREST

Professor Laing reports grants from Australian National Health and Medical Research Council, during the conduct of the study. The rest of authors have declared that no conflict of interest exists.

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TABLE LEGENDS

Table 1. Clinical features

				Manual muscle testing according to the MRC scale									
	Carriers	Age at exam	Onset	Neck flexor	Finger ext	Knee ext	Knee flexor	Hanging big toe	Toes ext	Ankle dorsiflexor	Pes cavus	FVC/ Echocardiogram	Pheno type
A	I-1	79 y	13 y	2/5	2/5	2/5	2/5	Yes	0/5	0/5	Yes	np/np	Sev
	II-1	59 y	8 y	3/5	4/5	4/5	5/5	Yes	2/5	0/5	Yes	N/np	Sev
	II-5	54 y	6 y	3/5	2/5	4/5	5/5	Yes	0/5	0/5	Yes	N/N	Sev
	II-6	52 y	6 y	3/5	3/5	3/5	5/5	Yes	3/5	0/5	Yes	N/N	Sev
	II-8	49 y	5 y	4/5	4/5	5/5	5/5	Yes	4/5	2/5	Yes	np/np	Mod
	II-14	39 y	30 y	5/5	5/5	5/5	5/5	Yes	4/5	2/5	Yes	N/N	Mod
	III-2	38 y	-	5/5	5/5	5/5	5/5	No	5/5	5/5	Yes	np/np	A
	III-4	23 y	6 y	5/5	5/5	5/5	5/5	Yes	5/5	5/5	Yes	np/np	Mild
	III-8	15 y	8y	4/5 (T)	5/5	5/5	5/5	No	5/5	5/5	No	np/np	Mild
	III-14	11 y	-	5/5	5/5	5/5	5/5	No	5/5	5/5	No	np/np	A
	III-15	8 y	4y	4/5 (T)	5/5	5/5	5/5	No	5/5	5/5	No	np/np	Mild
B	II-3	54 y	6 y	4/5	5/5	5/5	5/5	Yes	2/5	0/5	No	N/N	Mod
	II-5	42 y	7 y	5/5	5/5	5/5	5/5	Yes	5/5	5/5	No	np/np	Mild
	III-1	28 y	-	5/5	5/5	5/5	5/5	No	5/5	5/5	No	np/np	A
	III-2	21 y	17 y	5/5	5/5	5/5	5/5	Yes	5/5	5/5	No	np/np	Mild

y: Years; T: Transient; Ext: Extensor; CK: Serum creatin kinase; Numbers indicate muscle strength according to MRC scale; FVC: Forced vital capacity; N: Normal; np: not performed; A: Asymptomatic; Mild: Mild phenotype defined by hanging big toe sign; Mod: Moderate phenotype defined by weakness of toes extension and ankle dorsiflexion $\pm$ finger extension; Sev: Severe phenotype defined by weakness of toes extension, ankle dorsiflexion and knee extension $\pm$ finger extension. Bold letters denote mildly affected patients who only self-reported frequent falls during infancy.

Table 2. Muscle MRI findings

	Patient/ Family	Gluteus major	Quadriceps	Sartorius	Gracilis	Tibialis anterior	Gastrocnemius
Affected patients	I.1/A	np	np	np	np	np	np
	II.1/A	np	np	np	np	np	np
	II.5/A	+	+	++	+	+++	N
	II.6/A	+	++ (asymmetric)	+	+	+++	N
	II.8/A	np	np	np	np	np	np
	II.14/A	+	N	+	+	++	N
	II.3/B	+	N	+	N	+++	+
Mild affected patients	III.4/A	N	N	+	N	N	N
	III.8/A	np	np	np	np	np	np
	III.15/A	N	N	N	N	N	N
	II.5/B	N	N	+	N	N	N
	III.2/B	N	N	N	N	N	N
Asymptomatic carriers	III.2/A	N	N	+	+	N	N
	III.14/A	N	N	N	N	N	N
	III.1/B	N	N	+	+	N	N

N: Normal; +: Mild fatty infiltration; ++: Moderate fatty infiltration; +++: Severe fatty infiltration; np: not performed

FIGURE LEGENDS

Figure 1. Pedigrees of the two families with Laing distal myopathy. Clinical examination and genetic study were performed in all available family members. Circles denote female members, squares male members, solid black symbols affected patients, solid dark grey symbols mildly affected patients, solid light grey symbols asymptomatic carriers, striped symbols not-carriers, squared symbols not-examined carrier, and white symbols not-studied family members.

Figure 2. Clinical features. The most severely affected patients showed distal weakness involving finger extension (**A**) with mild contractures (**B**), and quadriceps weakness with atrophy only in the most advanced stages (**C**). Neck flexor weakness was present in patients with moderate and severe phenotype (**D**). Intriguingly, two infantile carriers presented isolated neck flexor weakness in the physical exam only during childhood (**E**), and disappeared in the course of the disease (**F**). The hanging big toe sign was present in early stage as first clinical sign of the disease, even in mildly affected patients who self-reported no symptoms (**G**, III.2/Family B; **H**, II.5/Family B; **I**, III.4/Family A), in some cases very subtle (**G**). No clear correlation was observed between the overall stage of clinical phenotype and the severity of the hanging big toe (i.e. compare **H** [II.5/Family B] to **K** [II.6/Family A], and **I** [III.4/Family A] to **J** [II.3/Family B]).

Figure 3. Muscle histology. Muscle biopsies from patient II.14/Family A, tibialis anterior (**A-D**) and patient II.3/Family B, gastrocnemius (**E-H**) were stained with haematoxylin and eosin (**A, B, E** and **F**), succinate dehydrogenase (SDH) (**C** and **G**) and ATPase pH 4.5 (**D** and **H**). Atrophy, variability in fibre size, increased endomysial connective tissue, and necrotic fibers (arrow) (**A** and **E**), and groups of small angulated atrophic fibers with nuclear clumps (arrowheads) (**B** and **F**) were present in both cases. SDH staining shows prominent sarcoplasmic disorganization including core (black arrowhead), core with poor defined borders (open arrow), and multiminicore (black arrow) lesions (**C** and **G**). Type I predominance was observed (**D**). Scale bar is 50 μ m in all panels (**A, B, C, E, F** and **G** x 200 magnification; **D** and **H** x 100 magnification).

Figure 4. Muscle MRI. Muscle MRI findings of patients displaying severe (**A**, II.6/ Family A), moderate (**B**, II.14/ Family A) and mild (**C**, II.5/ Family B) clinical phenotype. Asymptomatic carriers can show mild sartorius muscle infiltration (**D**, III.1/ Family B) and mild affected patients normal MRI (**E**, III.2/ Family B). The vastus intermedius and lateralis muscles are involved only in severe stages (**A**). The tibialis anterior was the most severely degenerated muscle in symptomatic patients (**A-C**), and usually the only affected muscle in the lower legs. The most striking finding in our study was that the sartorius muscle (arrowhead) was the earliest involved, even in asymptomatic carriers (**D**), although it does not show evidence of significant progression (**A-C**).

Figure 5. Haplotype analysis in the two families carrying the *MYH7* p.R1560P mutation. Genomic position for genotyped microsatellite and SNP markers relative to the *MYH7* gene, according to UCSC Genome Browser, Human GRCh37/hg19 (A). Haplotypes for families A and B (B, C). Haplotype analysis using microsatellite markers *D14S50*, *D14S283*, *D14S990*, *D14S972*, *D14S64* and *D14S264* in all available family members revealed a different haplotype cosegregating with the mutation in each family. To narrow down the studied genomic interval, we genotyped six SNP markers, *rs365990*, *rs434273*, *rs2331979*, *rs2277475*, *rs7157716* and *rs11621360* close to or within the *MYH7* gene. The analysis of SNP genotypes revealed a shared haplotype for markers *rs365990*, *rs434273*, *rs2331979* and *rs2277475* in all carriers of the p.R1560P mutation that defined a common region of 306 Kb. The shared common haplotype is boxed. Genotype for mutation p.R1560P is denoted by a + (wild-type) and M (mutant) sign. A question mark indicates unknown genotype. Solid black symbols denote carriers of the *MYH7* p.R1560P mutation.

A

ID	Feb. 2009 (GRCh37/hg19)
D14S50	14_22355826-22358093
D14S283	14_22687415-22687784
D14S990	14_23585265-23588632
rs365990	14_23861811
rs434273	14_23872996
rs2331979	14_23882855
MYH7	14_23881947-23904865
rs2277475	14_23885695
rs7157716	14_23892888
rs11621360	14_23911407
D14S972	14_24347553-24347945
D14S64	14_24560091-24560488
D14S264	14_25279919-25280182

B

C