**Supp. Table S1. Clinical and histological characteristics of patients in 41 families with novel, recurrent, and previously published mutations in *TPM2* (corresponding references in Supp. Table S2)**

| **Clinical and histological data of patients with *TPM2* mutations** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Family** | **Patient ID, Gender** | **M** | **Diagnosis** | **Histological characteristics (Age)** | **Presentation (Age)** | **Mobility (Age)** | **Associated features** | **Respiration** |
| Fam 1* | 2693<br><br>M | Ala4Gly | NM | Selective type 2 atrophy, central pallor in many type 1 fibres (66 yrs) | Proximal muscle weakness with onset subsequent to diagnosis of hypothyroidism (66 yrs) | Ambulant (73 yrs) | Limb-girdle pattern of proximal weakness | Normal |
| Fam 2# | Fam A<br><br>F (mother), M (son) Two unaffected male sibs of son | Lys7del | NM | **Mother and son:** Numerous small rods, core-like regions (7 yrs), abundant internal nuclei, split fibres, angular atrophic fibres (22 yrs) **Son:** Mild fibre size disproportion (type 1 fibres 30% smaller in diameter). 55% type 1 fibres | **Mother and son:** Walking delayed (age uncertain) | **Mother and son:** Awkward gait, falls (early childhood) | **Mother and son:** Contractures of jaw, shoulder, hips and ankles, mild proximal leg weakness. **Son:** Hypertonia | **Mother and son:** Normal |

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| Fam 3# | Fam B<br><br>M (parents unaffected, no affected or unaffected siblings) | Lys7del | NM | Numerous small rods, core-like regions in 80% of fibres, type 1 fibre predominance (67% type 1 fibres), type 1 fibres 23% smaller than type 2 fibres (41 yrs) | Contractures (early childhood) | Marked fatigue and myalgias on exertion (in adulthood) | Contractures of jaw, hips, shoulders, finger flexors, ankles, neck extensors | Not known |
| Fam 4# | Fam C<br><br>F | Lys7del | NM | Numerous small rods, internal nuclei, split fibres, sarcomeric disarray, type 1 fibre predominance (80% type 1 fibres), type 1 fibres 16% smaller than type 2 fibres (53 yrs) | Contractures (birth) | Walking difficult (50 yrs), ambulation lost (74 yrs) | Proximal limb weakness, severe kyphoscoliosis, schizophrenia, congenital contractures of elbows, knees, ankles, later onset contractures of jaw, pectoral muscles, finger extensors, quadriceps, hamstrings | Not known |
| Fam 5# | Fam D<br><br>M | Lys7del | NM | Numerous small rods, type 1 fibre predominance (2 yrs) | Abnormal gait (infancy) | Walked (13 mo) | Mild proximal limb and distal leg weakness, scoliosis, epilepsy, jaw and ankle contractures | Not known |

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| Fam 6 | Fam E 1653<br><br>F | Lys7del | NM | - | Delayed motor milestones, toe walking (infancy) | Quadriceps weakness (34 yrs), ambulation lost (62 yrs) | Tendo-achilles releases, myalgias | Not known |
| Fam 7*# | 20-747<br><br>F | Lys7del | NM | Type 1 fibre predominance and hypotrophy, rods (17 yrs) | Joint contractures (early infancy), no weakness | Walked (16 mo) | Joint pain, limitation of movement amplitude including mouth | Snoring, no alteration of vital capacity |
| Fam 8# | Fam 1<br>M | Lys7del | CRM | Variation in fibre size with both atrophic and hypertrophic fibres, internalised nuclei, type 1 fibre predominance, large rods, cap-like clusters, cores (9 yrs) | Gait unsteadiness (early childhood) | Not known | Bilateral calf and anterior tibialis atrophy, weakness of plantar flexion, flexed toes while walking, inability to toe walk, contractures, pronounced difficulty with mouth opening (i.e. trismus), pseudocamptodactyly (3 family members) | Not known |
| Fam 9# | Fam 2<br>F | Lys7del | DA type 7 | Scattered atrophic fibres, type 1 fibre predominance, rods (5 and 32 yrs) | Distal arthrogryposis (at birth) | Limited ambulation, uses wheelchair (15 yrs) | Bilateral calcaneovalgus feet, right vertical talus, tight knee flexion, congenital hip dislocations | Normal |
| Fam 10# | Fam 3<br>F | Lys7del | DA type 7 | Type 1 fibre predominance, numerous rods, | Walking delayed (18–24 mo) | Toe walking, difficulties running, | Kyphosis, short trunk and neck, limited jaw opening, contractures | FVC >75% |

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| | | | | minicore-like areas | | gait difficulties (13 yrs) | of the wrists, shoulders, hips and ankles | |
| Fam 11# | Fam 4<br>F | Lys7del | DA<br>type 7 | Myofibre hypertrophy and atrophy, rods and myofibrillar disarray including Z-band streaming, increase in internalised nuclei (23 yrs) | Walked with straightened legs and had 'stiff posture' (childhood) | Increased pace of walking or stair climbing worsens the stiffness leading legs to 'lock up' | Limited opening of the jaw, high-arched palate, pseudocamptodactyly (23 yrs), and fifth digit clinodactyly, 4+/5 bilateral weakness of deltoids | Not known |
| Fam 12# | P7-F4<br>F | Asp14Val | NM | Not known | Not known | Not known | Mild achilles contractures,<br>mild axial, pelvic and lower leg weakness | Normal |
| Fam 13 | 20-625<br>F | Asp14Val | NM<br><br><br><br><br><br><br>NM | **Index patient**: Lack of fibre differentiation, many dense rods (3 yrs 7 mo)<br>**Mother:** Lack of fibre differentiation, many dense rods (56 yrs)<br>**Sister:** no biopsy | **Index patient**: Diagnosed in childhood, progressive course of the disease<br>**Mother:** Progression of symptoms, requires assistance in most ADL (56 yrs)<br>**Sister:** | **Index patient**: Rising from floor, chair and stairs difficult, occupational therapy tests stable (30 yrs)<br>**Mother:** Electric wheelchair, decrease in manual strength and increase in fatigability | **Index patient**: Constipation and weight gain (2 y), slight scoliosis (30 yrs)<br>**Mother:** Proximal and distal weakness, cardiac ejection fraction 62% of normal, scoliosis (56 yrs)<br>**Sister:** Proximal and distal | **Index patient**: FVC 71% (30 yrs)<br>**Mother:** Hypercapnia (55 yrs), FVC 54% (56 yrs)<br>**Sister:** FVC 44% (19 yrs) |

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| | | | | | Diagnosed in childhood, no progression of peripheral symptoms (19 yrs) | (56 yrs)<br>**Sister:**<br>No progression, rising from floor, chair and stairs difficult, walking at normal pace (19 yrs) | weakness, lumbago, cardiac ejection fraction 60% of normal (19 yrs) | |
| Fam 14 | **Daughter:**<br>F<br><br>**Mother:**<br>F | Glu41Lys | NM<br>Cap | **Daughter:**<br>Type 1 fibre predominance (uniformity), caps but no rods, thick filaments partly lacking (26 yrs)<br>**Mother:** Type 1 fibre predominance (uniformity), no rods (32 yrs)<br><br>Primary myopathy with abnormal variability in fibre size, caps and numerous rods (57 yrs) | **Daughter:**<br>Hypotonia and feeding difficulties with poor sucking in infancy. Delayed motor milestones<br>**Mother:**<br>Respiratory insufficiency, delayed motor milestones, slowly progressive muscle weakness | **Daughter:**<br>Able to walk short distances only (35 yrs)<br>**Mother:**<br>Able to walk without support (57 yrs) | **Daughter:**<br>Moderate muscle weakness of proximal and distal muscles, neck flexors and facial muscles, myopathic facies, ptosis, scoliosis<br>**Mother:**<br>Moderate muscle weakness of proximal muscles, neck flexors and facial muscles, long narrow face, high-arched palate, micrognathia and dysphagia | Nocturnal non-invasive ventilation (mother and daughter)<br><br>**Daughter:**<br>FVC 41%<br><br>**Mother:**<br>FVC 42% |
| Fam 15 | 20-561 | Glu41Lys | CFTD | Fibre size | Severe neonatal | Achieved walking | Weakness of neck | Asymptomatic |

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| | F | | | disproportion (14 mo) | hypotonia, facial hypomimia | (2 yrs), able to run | flexors, rigid spine, mild facial hypomimia | |
| Fam 16 | M | Lys49del | Cap | Type 1 fibre predominance, variability in fibre size, no rods, intermyofibrillar coarse-meshed pattern (15 yrs)<br><br>Cap structures, disorganisation of myofibrils, partial loss of thick filaments (30 yrs) | Difficulty in sitting without support (8 mo) | Achieved walking but frequent falls (12 mo), lost ability to walk long distances (12 to 15 yrs), able to walk short distances (42 yrs) | Diffuse symmetric muscle weakness, waddling gait, Gowers' sign, kyphoscoliosis, high-pitched voice | Restricted vital capacity |
| Fam 17 | M | Gly52dup | Cap | Type 1 fibre predominance (uniformity), variability in fibre size, coarse-meshed intermyofibrillar network, cap structures, jagged Z lines (3 yrs) | Hypotonia (3 mo), delayed motor milestones | Achieved walking (17 mo), muscle weakness and difficulty walking and running (2 yrs), later walking and running more easily (5 yrs) | Speech nasal and dysarthric, myopathic facies, lumbar lordosis, Gowers' sign, weakness of neck and trunk extensors | Vital capacity normal |
| Fam 18 | M | Ser61Pro | CFTD | Fibre size | Generalised | Achieved | Mild facial weakness, | Normal |

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| | | | | disproportion (type 2 fibres 54% larger), type 1 fibre predominance (8 mo) | hypotonia (6 wks), delayed motor milestones | walking (23 mo), runs, jumps and climbs (3 yrs) | tendency to drool, mild proximal limb weakness | |
| Fam 19*# | F | 240+2T>G | DA<br>CM | Not known | Distal arthrogryposis and weakness (at birth) | Able to roll over and maintain sitting position if placed there but cannot sit on her own, does not crawl, lifts her head poorly in prone, is unable to raise her arms above shoulder height (13 mo) | Distal arthrogryposis with congenital contractures of ankles, fingers, and thumbs, tethered spinal cord, vertebral segmentation anomaly, dysmorphic facial features | Normal |
| Fam 20*# | F | 240+5G>A | DA | Not known | Hypotonia, joint contractures, and bilateral club foot (at birth) | Can pull to standing and cruise along furniture but cannot walk without support (2 yrs) | Club foot repair (8 mo) | Dysphagia, reactive airway disease |
| Fam 21# | F, M<br>14 family members | Arg91Gly | DA1 | Not known | Features of the upper limbs: ulnar deviation, camptodactyly, hypoplastic | Major findings in the lower limbs: talipes equinovarus, calcaneovalgus | Triangular face, down-slanting palpebral fissures, attached ear lobules, small mouth, small | Not known |

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| | | | | | and/or absent flexion creases, and overriding fingers at birth | deformities, vertical talus, and metatarsus varus | mandible, high-arched palate, cervical webbing, short stature | |
| Fam 22* | 20-601<br><br>F | Gln93His | CM<br>CFTD | Type 1 fibre predominance and hypotrophy, mild focal endomysial fibrosis | Neonatal hypotonia with respiratory difficulties | Delayed motor milestones, walking with support (3.5 yrs) | Axial and peripheral hypotonia, generalised muscle weakness, scoliosis, contractures of fingers, hips, sural triceps | Nocturnal non-invasive ventilation (3 yrs), tracheostomy (4 yrs) |
| Fam 23# | 5932, 5933<br>**Mother:**<br>F<br>**Son:**<br>M | Glu117Lys | NM<br>CFTD | **Mother and son:**<br>Type 1 fibre predominance and hypotrophy, no definite rods or caps | **Son:** Feeding difficulties, severe hypotonia at birth | **Mother:** Never able to run<br><br>**Son:** Delayed motor milestones, achieved ambulation | **Mother:** Myopathic facies, high-arched palate, neck flexors very weak, limb involvement asymmetric<br><br>**Son:** Facial weakness only mild, ankle contractures and a squint | **Son:** Normal respiration |
| Fam 24 | F | Glu117Lys | CFTD | Type 1 fibres small (medium diameter 18.2 µm) comprising 58% of fibres, type 2 fibres normal (mean diameter 36.9 µm) comprising | Hypotonia at birth, failure to thrive | Ambulatory (16 yrs) | Scoliosis, ptosis, micrognathia | Normal |

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| | | | | 42% of fibres | | | | |
| Fam 25 | F | Glu122Lys | CM | Type 1 fibre predominance, type 1 fibre hypotrophy, jagged Z lines (1 yr) | Feeding difficulties, poor weight gain, general muscle weakness, extreme head lag, bell-shaped thorax (4 mo) | Walks indoors, uses wheelchair for longer distances (7 yrs) | Proximal and axial muscles more severely affected than distal, neck flexors and spinal muscles weak, signs of scoliosis | Not known |
| Fam 26*# | 20-393<br>M | Lys128Glu | CFTD | Type 1 fibre predominance (1 yrs) | Hypotonia at birth | Walking achieved (2 yrs) | Scoliosis, mild weakness of neck flexors, deltoids, finger extensors of hands, minimal contractures of fingers and Achilles tendons | Asymptomatic |
| Fam 27# | F | Arg133Trp | DA2B | Type 1 fibre predominance | Distal joint contractures at birth | Not known | Progressive muscle weakness in proximal and distal muscles, especially hands and feet, high-arched palate, short neck, scoliosis | Not known |
| Fam 28# | 3603<br>F | Arg133Trp | NM | Rods | Congenital arthrogryposis, progressive weakness (14 yrs) | Barely ambulatory due to pes equinus (46 yrs) | Ptosis, short stature, symmetrical generalised weakness, contractures of fingers, wrists, elbows, ankles, jaw | FVC 59% of predicted value |
| Fam 29* | M | Arg133Pro | CFTD | Marked | Severe hypotonia | Severe | Facial weakness, high- | Tracheostomy, |

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| | | | | variation in fibre size, many small rounded fibers | and respiratory failure (at birth) | weakness, some antigravity movement of extremities | arched palate | mechanical ventilation |
| Fam 30 | **Daughter:**<br>291-A<br>F<br><br><br><br>**Father:**<br>291-B<br>M | Glu139del<br><br><br><br><br>Glu139del | NM, Cap<br><br><br><br><br>NM, Cap | **Daughter:**<br>Rods, cap structures, coarse intermyofibrillar network,<br>type 1 fibre predominance (15 yrs)<br>**Father:**<br>Rods, cap structures,<br>type 1 fibre predominance (14yrs) | **Daughter:**<br>Slowly progressive muscle weakness (first decade)<br><br><br>**Father:**<br>Moderate muscle weakness | **Daughter:**<br>Walked (13 m), normal development since 2 yrs, lost ability to walk (13 yrs)<br>**Father:**<br>Normal motor milestones | **Daughter:**<br>Diffuse weakness, long narrow face, scoliosis, rigid spine<br><br>**Father:**<br>Elongated face, high-arched palate, rigid spine mild scoliosis | **Daughter:**<br>Respiratory insufficency leading to hypercapnic coma.<br>FVC 60 % of predicted value |
| Fam 31 | F | Glu139del | CFTD | Fibre size disproportion and type 1 predominance | Birth | Able to walk | Elongated face, high-arched palate, cervical scoliosis | FVC 15 % of predicted value |
| Fam 32 | 3073<br><br>M | Glu139del | Cap<br>CFTD | Type 1 fibre hypotrophy, type 2 hypertrophy (12 yrs)<br><br>As previous biopsy, | Moderately hypotonic at birth, delayed motor milestones | Achieved walking (24 m), able to walk for short distances, never able to run, difficulties in climbing stairs | Myopathic facies, high-arched palate, ptosis, webbed neck, dysarthria, hyperlordosis, small muscle bulk, generalised muscle weakness, hypotonia | Acute respiratory insufficiency, hypoventilation, nosocomial pneumonia, BIPAP ventilation |

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| | | | | additional cap structures mostly in type 1 fibres (33 yrs) | | (33 yrs) | (11 yrs), weakness of facial muscles, knee flexors, shoulder girdle, ankle dorsiflexors, toe extensors (36 yrs) | initiated (33 yrs) |
| Fam 33 | F | Glu139del | Cap | Type 1 fibre hypotrophy and predominance, type 2A fibre hypertrophy, irregular Z lines, caps in 4% of fibres (10 yrs) | Poor weight gain, hypotonia, weakness, poor head control (4 mo) | Achieved walking (18 mo) and running in childhood but was slow and tired quickly, toe walking (10 yrs) | Mild facial weakness, nasal voice, high-arched palate, long thin face, mild micrognathia, generalised muscle wasting, scoliosis, cardiac involvement | Central hypoventilation, restrictive lung disease (13 yrs), FVC 46% (14 yrs) |
| Fam 34 | 3273<br>F | Glu139del | Cap<br>CFTD | Few rods (9 yrs) | Congenital, delayed motor milestones | Ambulant (39 yrs) | Myopathic facies, neck flexor weakness, ptosis, mild scoliosis, proximal muscles weaker than distal, symmetrical hyperlaxity of elbows | Not known |
| Fam 35* | 3143<br>F | Leu143Pro | NM<br>CFTD | Not known | No spontaneous antigravity movements, later improvement, distal weakness of lower limbs | Ambulant (40 yrs) | Mother and two sibs have similar weakness pattern | Night-time ventilation (18 yrs) |
| Fam 36 | 1331 | Gln147Pro | NM | Type 1 fibre | Walking delayed | Walking | Facial and neck flexor | Night-time |

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| | F | | Cap | predominance and selective hypotrophy, numerous muscle fibres with rods in cap formations and also caps without rods (50 yrs) | (2 yrs), never able to run | difficulty (12 yrs), ambulatory (34 yrs), uses wheelchair (48 yrs) | weakness only mild, bilateral ptosis, slight scapulae alatae, scoliosis, generalised mild muscle weakness, proximal more than distal, joint hyperlaxcity | ventilation (28 yrs) |
| Fam 37# | 3653<br><br>F | Leu148Pro | NM | Abundant atrophic fibres and rods, type 1 fibre predominance (44 yrs) | Subclinical weakness in childhood, presented with muscle weakness subsequent to infection (44 yrs) | Age at walking uncertain, ambulant with waddling gait (58 yrs) | Proximal muscles weaker than distal, Achilles contractures | Not known |
| Fam 38 | M | Ala155Val | Cap | Fibre size disproportion (type 2 fibres 59% larger than type1), type 1 fibre predominance, cap structures seen at EM (3 mo) | Weak fetal movements, severe neonatal hypotonia, frog-like posture, weak cry, poor suck | Slowly developed head control, able to roll over (12 mo), and sit (15 mo) | Generalised weakness with antigravity movements, mild ptosis | Resuscitation for respiratory insufficiency (birth), night-time ventilation (18 mo) |
| Fam 39# | **Mother:** F **Daughter:** F | Glu181Lys | NM | **Mother:** Mild fibre size variability | **Mother:** Short neck, elbow contractures (birth) | Not known | **Mother and daughter:** Temporomandibular, elbow, finger and | **Mother and daughter:** Normal |

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| | | | | (32 yrs)<br>**Daughter:**<br>Not available | **Daughter:**<br>Arthrogryposis (elbow)<br>torticollis, short neck<br>(birth) | | ankle contractures, stiffness of spine, knees and shoulders | |
| Fam 40 | F | Asn202Lys | Cap | Variability of fibre size, near uniformity of type 1 fibres, few cap structures, coarse-meshed intermyofibrillar network, jagged Z lines (5 yrs) | Weak fetal movements, hypotonia and insufficient ventilation at birth, fed via naso-gastric tube for 6 months, delayed motor milestones | Able to walk, able to walk on toes but not on heels | Facial diplegia, bilateral ptosis, muscle weakness severe in face and moderate in axial muscles, limited ocular movement, neck flexors weak, lumbar hyperlordosis, mild scoliosis, voice low-pitched and nasal | Normal |
| Fam 41 | M | Gln210Stop | NM Escobar syndrome | Even distribution of fibre types and sizes, rods (14% of fibres), slight type 1 predominance (60% to 40%) (2 yrs) | Major hypotonia, distal amyotrophy, delayed motor milestones | Standing achieved, no independent walking (6 yrs) | Consanguineous parents,<br>scoliosis, neither head nor sitting control, multiple pterygia affecting neck, axillar and antecubital area, ptosis | No respiratory or swallowing difficulties |

Nucleotide numbering according to the *TPM2* cDNA sequence with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence NM_003289.3. Amino acid coordinates are provided relative to NP_003280.2, the ATG translation initiation codon is codon 1. Abbreviations: NM, nemaline myopathy; Cap, cap myopathy; CFTD, congenital fibre type disproportion; DA, distal arthrogryposis; CRM, core rod myopathy; CM, undefined congenital myopathy; spl, splice site mutation; * new mutation; # gain-of-function mutation resulting in hypercontractile phenotypes.

**Supp. Table S2. Detailed description of novel, recurrent and previously published *TPM2* mutations in 41 families**

| Family No. | G | Disease | Location | Isoforms | Mode of inheritance | Mutation(s) in cDNA RefSeq NM003289 | Altered protein site and predicted effect | L | PolyPhen-2 / FATHMM prediction | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| Fam 1* | M | NM | exon 1a | muscle | AD *de novo* | c.8C>G | p.Ala3Gly | c | Benign / Damaging | Present study |
| Fam 2 # | M, F | NM | exon 1 | muscle | AD *de novo* | c.19_21delAAG | p.Lys7del | g | - | Mokbel *et al*. 2013 |
| Fam 3# | M | NM | exon 1 | muscle | AD *de novo* | c.19_21delAAG | p.Lys7del | g | - | Mokbel *et al*. 2013 |
| Fam 4 # | M, F | NM | exon 1 | muscle | AD *de novo* | c.19_21delAAG | p.Lys7del | g | - | Mokbel *et al*. 2013 |
| Fam 5 # | M | NM | exon 1 | muscle | AD *de novo* | c.19_21delAAG | p.Lys7del | g | - | Mokbel *et al*. 2013 |
| Fam 6 # | F | NM | exon 1 | muscle | AD *de novo* | c.19_21delAAG | p.Lys7del | g | - | Mokbel *et al*. 2013 |
| Fam 7 # | F | NM | exon 1a | muscle | AD *de novo* | c.19_21delAAG | p.Lys7del | g | - | Present study |
| Fam 8 # | M | CRM | exon 1a | muscle | AD | c.19_21delAAG | p.Lys7del | g | - | Davidson *et al*. 2012 |
| Fam 9 # | F | DA type7 | exon 1a | muscle | AD | c.19_21delAAG | p.Lys7del | g | - | Davidson *et al*. 2012 |
| Fam 10 # | F | DA type7 | exon 1a | muscle | AD | c.19_21delAAG | p.Lys7del | g | - | Davidson *et al*. 2012 |
| Fam 11 # | F | DA type7 | exon 1a | muscle | AD | c.19_21delAAG | p.Lys7del | g | - | Davidson *et al*. 2012 |
| Fam 12 # | F | NM | exon 1a | muscle | AD *de novo* | c.41A>T | p.Asp14Val | g | Possibly damaging / Damaging | Jarraya *et al*. 2012 |

| **Family No.** | **G** | **Disease** | **Location** | **Isoforms** | **Mode of inheritance** | **Mutation(s) in cDNA RefSeq NM003289** | **Altered protein site and predicted effect** | **L** | **PolyPhen-2 / FATHMM prediction** | **Reference** |
|---|---|---|---|---|---|---|---|---|---|---|
| Fam 13 | F | NM | exon 1a | muscle | AD | c.41A>T | p.Asp14Val | g | Possibly damaging / Damaging | Present study |
| Fam 14 | F | NM Cap | exon 2 | muscle | AD | c.124G>A | p.Glu41Lys | f | Benign / Tolerated | Tajsharghi *et al*. 2007 |
| Fam 15 | F | CFTD | exon 2 | muscle | AD | c.124G>A | p.Glu41Lys | f | Benign / Tolerated | Present study |
| Fam 16 | M | Cap | exon 2 | muscle | AD *de novo* | c.144_146 delGAA | p.Lys49del | g | - | Ohlsson *et al*. 2008 |
| Fam 17 | M | Cap | exon 2 | muscle | AD *de novo* | c.153_155 dupGGG | p.Gly52dup | c | - | Ohlsson *et al*. 2008 |
| Fam 18 | M | CFTD | exon 2 | muscle | AD *de novo* | c.181T>C | p.Ser61Pro | e | Probably damaging / Tolerated | Clarke *et al*. 2012 |
| Fam 19* # | F | DA CM | intron 2 | muscle | AD *de novo* | c.240+2T>G | spl | | - | Present study |
| Fam 20* # | F | DA | intron 2 | all | AD *de novo* | c.240+5G>A | spl | | - | Present study |
| Fam 21 # | F, M | DA1 | exon 3 | all | AD | c.271C>G | p.Arg91Gly | g | Benign / Damaging | Sung *et al*. 2003 |
| Fam 22* | F | CM | exon 3 | all | AD *de novo* | c.279G>C | p.Gln93His | b | Probably damaging / Damaging | Present study |
| Fam 23 # | M | NM CFTD | exon 3 | all | AD *de novo* | c.349G>A | p.Glu117Lys | e | Possibly damaging / Damaging | Donner *et al*. 2002 (41) Brandis *et al*. 2008 (42) |
| Fam 24 | F | CFTD | exon 3 | all | AD | c.349G>A | p.Glu117Lys | e | Possibly | Present study |

| **Family No.** | **G** | **Disease** | **Location** | **Isoforms** | **Mode of inheritance** | **Mutation(s) in cDNA RefSeq NM003289** | **Altered protein site and predicted effect** | **L** | **PolyPhen-2 / FATHMM prediction** | **Reference** |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | damaging / Damaging | |
| Fam 25 | F | CM | exon 3 | all | AD | c.364G>A | p.Glu122Lys | c | Possibly damaging / Damaging | Tajsharghi *et al*. 2012 |
| Fam 26*# | M | CFTD | exon 4 | all | AD *de novo* | c.382A>G | p.Lys128Glu | b | Probably damaging / Damaging | Present study |
| Fam 27 # | F | DA2B | exon 4 | all | AD *de novo* | c.397C>T | p.Arg133Trp | g | Possibly damaging / Damaging | Tajsharghi *et al*. 2007 |
| Fam 28 # | F | NM DA CFTD | exon 4 | all | AD *de novo* | c.397C> T | p.Arg133Trp | g | Possibly damaging / Damaging | Present study |
| Fam 29* | M | CFTD | exon 4 | all | AD | c.398G>C | p.Arg133Pro | g | Probably damaging / Damaging | Present study |
| Fam 30 | F, M | NM Cap | exon 4 | muscle | AD (mosaic father) | c.415_417delGAG | (p.Glu138del) p.Glu139del | e | - | Tasca *et al*. 2013 |
| Fam 31 | F | CFTD | exon 4 | muscle | AD *de novo* | c.415_417delGAG | p.Glu139del | e | - | Present study |
| Fam 32 | M | Cap CFTD | exon 4 | all | AD *de novo* | c.415_417delGAG | p.Glu139del | f | - | Lehtokari *et al*. 2007 |
| Fam 33 | F | Cap | exon 4 | all | AD *de novo* | c.415_417delGAG | p.Glu139del | f | - | Clarke *et al*. 2009 |
| Fam 34 | F | Cap | exon 4 | all | AD | c.415_417delGA | p.Glu139del | f | - | Present study |

| Family No. | G | Disease | Location | Isoforms | Mode of inheritance | Mutation(s) in cDNA RefSeq NM003289 | Altered protein site and predicted effect | L | PolyPhen-2 / FATHMM prediction | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| | | CFTD | | | *de novo* | G | | | | |
| Fam 35* | F | NM CFTD | exon 4 | all | AD | c.428T>C | p.Leu143Pro | c | Possibly damaging / Damaging | Present study |
| Fam 36 | F | NM Cap | exon 4 | all | AD *de novo* | c.440A>C | p.Gln147Pro | g | Probably damaging / Tolerated | Donner *et al.* 2002 Brandis *et al*. 2008 |
| Fam 37*# | F | NM adult onset | exon 4 | all | AD *de novo* | c.443T>C | p.Leu148Pro | a | Possibly damaging / Damaging | Present study |
| Fam 38 | M | Cap | exon 4 | all | AD *de novo* | c.464C>T | p.Ala155Val | a | Probably damaging / Damaging | Clarke *et al*. 2012 |
| Fam 39 # | F | NM | exon 5 | all | AD | c.541G>A | p.Glu181Lys | f | Probably damaging / Tolerated | Jarraya *et al.* 2012 |
| Fam 40 | F | Cap | exon 6 | | AD *de novo* | c.606C>G | p.Asn202Lys | f | Benign / Tolerated | Ohlsson *et al*. 2008 |
| Fam 41 | M | NM Escobar syndrome | exon 6b | muscle | AR | c.628C>T | p.Gln210Stop | g | - | Monnier *et al*. 2009 |

PolyPhen-2 and FATHMM predictions indicate the possible pathogenicity of the missense mutations. The possible pathogenicity of the mutations was assessed with PolyPhen-2, version 2.2.2, HumVar option, and FATHMM version 2.3 with the prediction algorithm weighted for human mutations and disease ontology as the option for phenotypic associations. Nucleotide numbering according to the *TPM2* cDNA sequence

with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence NM_003289.3. Amino acid coordinates are provided relative to NP_003280.2, the ATG translation initiation codon is codon 1. Abbreviations: NM, nemaline myopathy; Cap, cap myopathy; CFTD, congenital fibre type disproportion; DA, distal arthrogryposis; CRM, core rod myopathy; CM, undefined congenital myopathy; G, gender; AD, autosomal dominant; AR, autosomal recessive; L, location in heptad repeat; spl, splice site mutation; * new mutation; # gain-of-function mutation resulting in hypercontractile phenotypes.

**Supp. Table S3. Clinical and histological characteristics of patients in 35 families with novel, recurrent and previously published mutations in *TPM3* (corresponding references in Supp. Table S4)**

| **Clinical and histological data of patients with *TPM3* mutations** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Family** | **Patient ID, Gender** | **Mutation** | **Diagnosis** | **Histological characteristics (Age)** | **Presentation (Age)** | **Mobility (Age)** | **Associated features** | **Respiration** |
| Fam 1 | BOS 126-2<br><br>F<br><br><br><br><br><br><br>126-1<br><br>M | Ala4Val<br><br><br><br><br><br><br><br>Ala4Val | CFTD<br><br><br><br><br><br><br><br>CFTD | **Mother:**<br>Fibre size disproportion (type 1 fibres 33% smaller than type 2 fibres), mild type 1 fibre predominance (57%) (29 yrs)<br>**Son :**<br>Fibre size disproportion (type 1 fibres 47% smaller than type 2 fibres), the majority of small fibres are of type 1 (16 mo) | **Mother:**<br>Diagnosed retrospectively (29 yrs), following diagnosis in her son<br><br>**Son :**<br>Poor feeding and hypotonia (birth) | **Mother:**<br>Walking, slow runner, fatigues easily, ambulatory (39 yrs)<br><br><br><br>**Son :**<br>Walked at 18-21 mo, able to run (4 yrs), ambulatory (14.5 yrs) | **Mother:**<br>Ptosis, myopathic facies, diffuse mild weakness, high-arched palate (39 yrs)<br><br><br><br>**Son :**<br>History of cryptorchidism, ADHD and language disorder, normal muscle tone, high- arched palate (5.5 yrs) | **Mother:**<br>Normal<br><br><br><br><br><br>**Son :**<br>Normal |
| Fam 2 | F, M<br>10 affected family members | Met9Arg | NM | Numerous rods in predominant type 1 fibres, marked variation in fibre size (5- | Weakness of the feet (<10 yrs) | Progressive weakness of the anterior tibial and ankle movement (<10 yrs), reflexes | Developing pes cavus, weakness of hip flexion, progressive wasting of the | Not known |

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| | | | | 120 µm) | | lost in lower limbs at late stages | lower limbs, mild proximal arm wasting, dysphagia | |
| Fam 3* | 3363<br><br>M | Ser88Phe | NM<br>Cap | Type 1 fibres small, a few large type 1 and 2 fibres<br>(54, 56, and 64 yrs) | Myalgia (40 yrs), weakness (> 50 yrs) | Ambulant (70 yrs) | Muscle soreness, no facial weakness, asymmetry, lower limbs weaker than upper, progression, proximal and distal weakness | FVC normal |
| Fam 4 | BOS 913-1<br><br>F | Arg91Pro | CFTD | Type 1 hypotrophy<br>(6 mo) | Hypotonia and bilateral facial paresis (2 mo) | Non-ambulatory (14 mo) | Moderate diffuse weakness, absence of neck control, dysarthria | Neonatal tachypnea and seasaw breathing |
| Fam 5* | 20-799<br><br>M | Arg91Cys | CM | Type 1 fibre hypotrophy , 5% internalised nuclei | Ventilatory compromise<br>(56 yrs) | Moderate scapular and pelvic weakness (61 yrs) | Hypoacusis | Non-invasive ventilation<br>(56 yrs) |
| Fam 6 | F, M<br>(seven affected family members over 3 generations) | Leu100Met | CFTD | Fibre size disproportion (type 1 fibres 50 to 65 % smaller than type 2 fibres),<br>type 1 fibre predominance<br>(3, 32, 36 yrs in different family members) | Hypotonia, delayed motor milestones in infancy | Running (16 yrs), independent walking (40> yrs) | Mild to moderate weakness in scapular region, slowly progressive generalised weakness, proximal more than distal, facial weakness, high-arched palate, absent reflexes, protuberant eyes | From asymptomatic to requiring nocturnal non-invasive ventilation<br>(41 yrs)<br><br>FVC 88%<br>(13 yrs) |
| Fam 7* | F | Leu100Val | CFTD | **Mother:**<br>Type 1 fiber predominance | **Daughter:**<br>Neonatal hypotonia (generalised), head | **Daughter:**<br>Achieved walking (14 mo) and | **Daughter:**<br>Mild proximal weakness involving | Normal |

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| | | | | and fiber smallness, all muscle fibers have peripherally located nuclei (23 yrs) | lag, areflexia<br><br>**Mother:** Hypotonic since infancy | running (2 yrs)<br><br>**Mother:** Hypotonic since infancy, currently able to run with difficulty (28 yrs) | trunk, shoulder and hip girdle, minimal involvement of facial and neck muscles, no progression, reflexes absent apart from ankle jerks (1/4), high-arched palate<br><br>**Mother:** Mild finger flexor contractures facial weakness, high-arched palate, protruding eyes, mild scapular winging, mild shoulder and hip girdle weakness, wrist and finger extensor weakness (asymmetrical) | |
| Fam 8* | 20-607<br><br>F | Glu151Ala | Cap | Type 1 fibre uniformity, irregular intermyofibrillar network, caps | Weakness of neck flexor (3 mo) | Achieved walking (21 mo), able to climb stairs, frequent falls | Axial weakness, neck flexor weakness, mildly rigid spine, lumbar hyperlordosis, mild proximal weakness | Normal |
| Fam 9 | F | Ala156Thr | NM | Type 1 fibre | Muscle weakness | Not known | Elongated face, | Restricted vital |

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| | | | | predominance, 80% of fibres contain rods bodies (54 yrs) | (early childhood) | | mild facial weakness, high-arched palate, generalised muscle weakness and wasting of proximal muscles (57 yrs) | capacity (48%), nocturnal continuous positive airway pressure ventilation (54 yrs) |
| Fam 10 | 1963<br><br>F | Arg168Cys | NM | Type 1 fibres only<br>(34 yrs) | Congenital, floppy infant, milestones not delayed | Walking with difficulty<br>(44 yrs) | Myopathic facies, neck flexor weakness, high-pitched voice, proximal and distal weakness, asymmetry, scoliosis | FEV 55 % |
| Fam 11 | F | Arg168Cys | CFTD | Fibre type differentiation poor (6 yrs)<br><br>Fibre size disproportion (type 1 fibres 77% smaller than type 2 fibres), type 1 fibre predominance (19 yrs) | Poor head control, respiratory infections in infancy | Running in childhood, walks, stairs difficult (32 yrs) | Moderate weakness of neck flexion, severe kyphoscoliosis | Nocturnal non-invasive ventilation (19 yrs) |
| Fam 12 | BOS1109-1<br><br>F | Arg168Cys | CFTD | Not known | Hypotonia in infancy, high arched palate, | Ambulatory (36 yrs), able to walk but uses wheelchair | Scoliosis, long, narrow face, hyperthyroidism, | Tracheostomy |

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| | | | | | narrow face | at work | episodic tachycardia, moderate proximal and distal weakness, foot drop | |
| Fam 13 | 20-837<br><br>M | Arg168Cys | NM<br>Cap | Rods and caps<br>(19 yrs) | Transient axial hypotonia (3 mo), walking (14 mo), myopathy (19 yrs) | Walks with support, no wheelchair | Kyphosis, bilateral ptosis | First respiratory decompensation (32 yrs) |
| Fam 14 | F | Arg168Cys | CFTD | Variation in fibre size with some small type 1 fibres | Weakness of the feet , frequent falls (3 years) | Ambulant, slow runner<br>(9 yrs) | Developing pes cavus, some distal laxity, mild distal weakness (L>R) | Normal |
| Fam 15 | F | Arg168Gly | CFTD | Fibre size disproportion (type 1 fibres at least 50% smaller than type 2 fibres),<br>type 1 fibre predominance<br>(10 yrs) | Slow running<br>(8 yrs) | Able to run (9 yrs) | Mild facial and neck flexor weakness | FVC 50%<br>(12 yrs) |
| Fam 16 | F | Arg168His | CFTD | **Mother:**<br>Fibre size disproportion (56%),<br>type 1 fibre predominance (68%)<br>(40 yrs) | **Mother:**<br>Generalised muscle hypotonia (at birth)<br><br>**Daughter:** Bilateral talipes (at birth) | **Mother:**<br>Achieved walking (2 yrs)<br><br>**Daughter:**<br>Achieved walking (15 mo), running (childhood) | Neck flexor weakness (birth) | Asymptomatic |

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| Fam 17 | M | Arg168His | CFTD | Fibre size disproportion (44%), type 1 fibre predominance (46%) (6 mo) | Severe generalised weakness, hypotonia, weak cry, poor suck (at birth) | Achieved walking (3 yrs), climbing stairs (4.5 yrs) | Hypotonic with absent reflexes, myopathic facies, high-arched palate, mild drooling, mild weakness in distal muscles, poor finger movements | Asymptomatic |
| Fam 18 | F, M (daughter and father) | Arg168His | NM CFTD | **Daughter:** Rare rods in small fibres (20 yrs)<br><br>**Father:** Fibre size disproportion (type 1 fibres 60-72% smaller than type 2 fibres), type 1 fibre predominance (56 yrs) | **Daughter:** Hypotonia in infancy | **Daughter:** Achieved walking (18 mo). Runs at age 20 yrs<br><br>**Father:** Achieved walking (5 yrs), able to run (15 – 35 yrs), now walks slowly (56 yrs) | **Daughter:** Proximal limb girdle weakness, prominent weakness of neck flexion and ankle dorsiflexion, mild facial weakness, mild ptosis | **Daughter:** FVC 57% (20 yrs)<br><br>**Father:** Nocturnal non-invasive ventilation (55 yrs) |
| Fam 19 | M | Arg168His | NM | Variation in fibre size, type 1 fibre predominance, rods in type 1 fibres in very low proportion (<1%) (53 yrs) | Poor physical performance in childhood | Started walking (17 mo), has never been able to run or jump, walks unaided (53 yrs) | Elongated face, very high-arched palate, badly implanted teeth, large protruding ears, need hand support to rise from chair (53 yrs) | FVC 55% (50 yrs) Nocturnal non-invasive ventilation (50 yrs) |

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| Fam 20 | M | Arg168His | Cap | Type 1 fibre hypotrophy, type 1 fibres 4 to 5 times smaller than type 2 fibres, no caps observed (7 yrs)<br><br>Type 1 fibres only, irregularity of fibre size with occasional centrally located nuclei, caps in 10-15% of fibres, coarse-meshed intermyofibrillar network (42 yrs) | Hypotonia in first months, delayed motor milestones, frequent falls and distal weakness of lower limbs (4 yrs) | Unable to run and difficulty in climbing stairs, walking distance not limited (42 yrs) | Flat feet, long, narrow face, high-arched palate, slight distal weakness of lower limbs (7 yrs), mild Gowers‘ sign, motor weakness predominantly distal | - |
| Fam 21 | BOS 247-4<br><br>F | Arg168His | CFTD | Type 1 fibre hypotrophy (11 yrs) | Hypotonia, delayed motor milestones and ligamentous laxity (4 mo) | Achieved standing (18 mo), walking (19 mo), running (3 yrs), able to jump, but not very high | More axial than proximal weakness, scoliosis, fatigue, feeding difficulty, poor weight gain | BiPAP ventilation since 12 yrs |
| Fam 22 | BOS 343-1<br><br>M | Arg168His | NM | Fibre size disproportion (type1/type2 0.48), type 1 fibre predominance and hypotrophy, | Respiration difficulty and weakness (58 yrs) | Ambulatory until death (66 yrs) | Mild scoliosis, hip contracture, difficulty raising arms and holding head, hypothyroidism | Occasional nocturnal oxygen (since 58 yrs) |

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| | | | | numerous rods in type 1 fibres (59 yrs) | | | | |
| Fam 23 | F (Mother and daughter) | Arg168His | CFTD | **Mother and daughter:** Fibre size disproportion, type 1 fibre predominance (size averaging 30µm, type 2 fibre size averaging 80 µm) | **Mother and daughter:** Floppy from birth, motor delay<br><br>**Daughter:** born 38 wks elective caesarian, hypotonia and poor sucking (2 mo) | **Mother:** Achieved sitting (9 mo) and walking (3 yrs), never able to walk far, run or jump, ambulant, able to climb stairs<br><br>**Daughter:** Walked with zimmer (2.5yrs), never able to run jump or hop, remains ambulant and able to climb stairs, failure to gain weight despite adequate oral intake | **Mother:** Facial weakness, ptosis, axial weakness, proximal and distal weakness, legs weaker than arms (51 yrs)<br><br>**Daughter:** Ptosis, facial weakness, proximal weakness in arms and legs, high-arched palate, very thin (18 yrs) | **Mother:** Nocturnal non-invasive ventilation (42 yrs)<br><br>**Daughter:** FVC 60-70% (in childhood) |
| Fam 24 | F | Arg168His | CFTD | Variation in fibre size, type1 fibre hypotrophy | Persistent hypotonia, motor delay and generalized joint laxity (4 mo) | Walked at 18 months, runs (3 yrs) | Spinal rigidity and scoliosis, facial weakness, finger flexor tightness (12 yrs), myopathic facies, nasal voice, high arched palate (13 yrs) | FVC 63% (12 yrs), non-invasive ventilation (13 yrs) |
| Fam 25 # | M | Lys169Glu | CFTD | Fibre size | Hypotonia, mild | Slow runner (8 | Moderate facial | FVC 50% (8 |

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| | | | | disproportion (type 1 fibres 53% smaller than type 2 fibres), type 1 fibre predominance (1.4 yrs) | weakness in infancy | yrs), Gowers' manoeuvre | weakness, mild ptosis, pronounced neck flexor weakness | yrs) |
| Fam 26 | M | Glu174Ala | CFTD | Fibre size disproportion, type 1 fibre predominance, (2 yrs) | Hypotonia and feeding difficulties after birth | Walked (14 mo), never able to run or jump | Myopathic facies, high-arched palate, facial weakness and thin muscle bulk (7 yrs) | Nocturnal non-invasive ventilation (2 yrs) |
| Fam 27 | BOS 311-1<br>F | Glu241Lys | CFTD | Fibre size disproportion (type1/type2 0.48), type 1 fibre hypotrophy (5 mo) | Presented in utero with abnormal posture and respiration/feeding difficulty at birth, diffuse hypotonia and delayed motor milestones (6 mo) | Achieved walking at 5 yrs, walking with effort (9 yrs), unable to run | High-arched palate, dysarthria, mild hip contractures | Insufficient at birth, required oxygen within first few days, BiPAP ventilation since 8 yrs |
| Fam 28 | M | Arg245Gly | CFTD | Fibre size disproportion (type 1 fibres 70% smaller than type 2 fibres), type 1 fibre predominance (1.8 yrs) | Delayed motor milestones (3 mo) | Walking (3.5 yrs) | Moderate weakness of scapulae and neck muscles | Nocturnal non-invasive ventilation (3.5 yrs) |
| Fam 29* | 20-834<br>F | Arg245Ile | Cap | Type I fibre hypotrophy, caps | Severe generalized hypotonia and hyporeflexia, poor | Able to sit but not to stand up (19 mo) | Proximal weakness, pronounced in | Nocturnal hypercapnia (8 mo), |

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| | | | | | sucking and hypotrophia (at birth) | | pelvic girdle, no facial weakness, ocular movements normal | nocturnal non-invasive ventilation (2 yrs) |
| Fam 30* | 20-872<br>F | Thr252Lys | CM | Type 1 fibre predominance | Congenital hypotonia, delayed motor milestones | Walking (2 yrs 6 mo) | Scapular amyotrophy, lumbar lordosis, myopathic facies, patellar areflexia | Asymptomatic |
| Fam 31 | M | Gln32Stop | NM severe | Type 1 fibre hypotrophy (3-9 μm), type 2 fibres uniform in size (30–40 μm), slight predominance of type 2 fibres, rods present in type 1 fibres only | Motor development extremely delayed and impaired in infancy, hypotonic with no head control | Able to roll over in infancy, unable to lift head from prone position, unable to sit or stand unaided | Consanguineous parents. Cognitive and speech development normal, died with bradycardia and decreased levels of oxygen saturation (21 mo) | Deceased with decreased levels of oxygen saturation (21 mo) |
| Fam 32 | F | Stop285Ser | NM intermediate | Rods in type 1 fibres, type 1 fibre predominance, central nuclei, increased endomysial connective tissue (5 yrs) | Hypotonic with noisy breathing in infancy, abnormal gait, frequent falling, and inability to run (2 yrs) | Achieved crawling (9 mo) and walking (17 mo), loss of ambulation (5 yrs), uses wheelchair (6 yrs) | Long face with open mouth, severely impaired ankle dorsiflexion (3 y) | Not known |
| Fam 33 | BOS 313-1<br>F | Stop285Ser | CFTD | Fibre size disproportion (type 1 fibres | Hypotonia and haed lag (3 mo) | Non-ambulant, did not achieve walking | Severe weakness of upper and lower limbs, high-arched | Tracheostomy, ventilator-dependent |

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| | | | | 70% smaller than type 2 fibres), marked fibre size variation, occasional central nuclei in atrophic fibres (1 yr) | | (9.5 yrs) | palate, dysarthria, facial weakness, pectus excavatum (2.4 yrs) | (2.5 yrs) |
| Fam 34 and 35 | **Fam 31:** Two brothers: M **Fam 32:** Sister: F Brother: M | Stop285Asn | NM severe | **Fam 31:** Hypotrophic type 1 fibres with rods **Fam 32:** Type 1 fibres markedly smaller than the type 2 fibres (3 mo) | **Fam 31:** Contractures of the knees and ankles, delayed motor milestones **Fam 32:** Hypotonia in infancy, contractures of the knees and ankles, delayed motor milestones | **Fam 31:** Slow walking with waddling gate (2.5 yrs) **Fam 32:** Few steps with support and could lift hand to mouth but not arms completely (6 yrs) | Weakness of neck muscles, facial weakness, lack of head control, scoliosis observed during 1st yr | Nocturnal non-invasive ventilation (from 10 yrs) |

Nucleotide numbering according to the coding sequence of the *TPM3* cDNA reference sequence NM_152263.3. The first 2 ATG codons in the primary cDNA were both included according to the Human Genome Variation Society recommendations. Amino acid coordinates are provided relative to NP_689476.2, the ATG translation initiation codon is codon 1. Abbreviations: NM, nemaline myopathy; Cap, cap myopathy; CFTD, congenital fibre type disproportion; CM, undefined congenital myopathy; spl, splice site mutation; * new mutation; # gain-of-function mutation resulting in hypercontractile phenotypes.

**Supp. Table S4. Detailed description of novel, recurrent and previously published *TPM3* mutations in 35 families**

| Family No. | G | Disease | Location | Isoforms affected | Mode of inheritance | Mutation(s) in cDNA RefSeq NM152263.3 | Altered protein site and predicted effect | L | PolyPhen-2 / FATHMM prediction | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| Fam 1 | F, M | CFTD | exon1 | muscle | AD | c.11C>T | (p.Ala3Val) p.Ala4Val | C | Probably damaging / Damaging | Lawlor et al. 2010 |
| Fam 2 | F, M | NM | exon 1 | muscle | AD | c.26T>G | (p.Met8Arg) p.Met9Arg | A | Probably damaging / Damaging | Laing et al. 1995 |
| Fam 3* | M | NM Cap | exon 3 | all | AD | c.263C>T | (p.Ser87Phe) p.Ser88Phe | c | Probably damaging / Damaging | Present study |
| Fam 4 | F | CFTD | exon 3 | all | AD de novo | c.272G>C | (p.Arg90Pro) p.Arg91Pro | F | Probably damaging / Damaging | Lawlor et al. 2010 |
| Fam 5* | M | CM | exon 3 | all | Probable mosaicism | c.271C>T | (p.Arg90Cys) p.Arg91Cys | f | Probably damaging / Damaging | Present study |
| Fam 6 | F, M | CFTD | exon 3 | all | AD | c.298C>A | (p.Leu99Met) p.Leu100Met | A | Probably damaging / Damaging | Clarke et al. 2008 (Fam 4) |
| Fam 7* | F | CFTD | exon 3 | all | AD | c.298C>G | (p.Leu99Val) p.Leu100Val | a | Benign / Damaging | Present study |
| Fam 8* | F | Cap | exon 4 | all | AD *de novo* | c.452A>C | (p.Glu150Ala) p.Glu151Ala | c | Probably damaging / Damaging | Present study |
| Fam 9 | F | NM | exon 4 | all | AD | c.466G>A | (p.Ala155Thr) p.Ala156Thr | a | Probably damaging / | Kiphuth et al. 2010 |

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| | | | | | | | | | Damaging | |
| Fam 10 | F | NM | exon 5 | all | AD | c.502C>T | (p.Arg167Cys) p.Arg168Cys | F | Probably damaging / Damaging | Present study |
| Fam 11 | F | CFTD | exon 5 | all | AD *de novo* | c.502C>T | (p.Arg167Cys) p.Arg168Cys | F | Probably damaging / Damaging | Clarke et al. 2008 Fam 5 |
| Fam 12 | F | CFTD | exon 5 | all | AD | c.502C>T | (p.Arg167Cys) p.Arg168Cys | F | Probably damaging / Damaging | Lawlor et al. 2010 |
| Fam 13 | M | NM Cap | exon 5 | all | AD de novo | c.502C>T | (p.Arg167Cys) p.Arg168Cys | f | Probably damaging / Damaging | Present study |
| Fam 14 | F | CFTD | exon 5 | muscle | AD *de novo* | c.502C>T | (p.Arg167Cys) p.Arg168Cys | f | Probably damaging / Damaging | Present study |
| Fam 15 | F | CFTD | exon 5 | all | AD *de novo* likely | c.502C>G | (p.Arg167Gly) p.Arg168Gly | f | Probably damaging / Damaging | Clarke et al. 2008 (Fam 3) |
| Fam 16 | F | CFTD | exon 5 | all | AD | c.503G>A | (p.Arg167His) p.Arg168His | f | Benign / Damaging | Present study |
| Fam 17 | M | CFTD | exon 5 | all | AD *de novo* | c.503G>A | (p.Arg167His) p.Arg168His | f | Benign / Damaging | Present study |
| Fam 18 | F, M | NM CFTD | exon 5 | all | AD *de novo* | c.503G>A | (p.Arg167His) p.Arg168His | f | Benign / Damaging | Clarke et al. 2008 (Fam 6) Durling et al. 2002 |
| Fam 19 | M | NM | exon 5 | all | AD | c.503G>A | (p.Arg167His) | f | Benign / | Penisson- |

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| | | | | | | | p.Arg168His | | Damaging | Besnier et al. 2007 |
| Fam 20 | M | Cap | exon 5 | all | AD *de novo* | c.503G>A | (p.Arg167His) p.Arg168His | f | Benign / Damaging | De Paula et al. 2009 |
| Fam 21 | F | CFTD | exon 5 | all | AD de novo | c.503G>A | (p.Arg167His) p.Arg168His | f | Benign / Damaging | Lawlor et al. 2010 |
| Fam 22 | M | NM | exon 5 | all | AD de novo | c.503G>A | (p.Arg167His) p.Arg168His | f | Benign / Damaging | Lawlor et al. 2010 |
| Fam 23 | F | CFTD | exon 5 | all | AD | c.503G>A | (p.Arg167His) p.Arg168His (RYR p.Arg3539His) | f | Benign / Damaging | Klein et al. 2012 |
| Fam 24 | F | CFTD | exon 5 | all | AD | c.503G>A | (p.Arg167His) p.Arg168His | f | Benign / Damaging | Munot et al. 2010 |
| Fam 25# | M | CFTD | exon 5 | all | AD *de novo* | c.505A>G | (p.Lys168Glu) p.Lys169Glu | g | Probably damaging / Damaging | Clarke *et al*. 2008 (Fam 2) |
| Fam 26 | M | CFTD | exon 5 | all | probably *de novo* | c.521A>C | (p.Glu173Ala) p.Glu174Ala | f | Probably damaging / Damaging | Munot et al. 2010 |
| Fam 27 | F | CFTD | exon 8 | all | AD de novo | c.721G>A | (p.Glu240Lys) p.Glu241Lys | b | Probably damaging / Damaging | Lawlor et al. 2010 |
| Fam 28 | M | CFTD | exon 8 | all | AD *de novo* | c.733A>G | (p.Arg244Gly) p.Arg245Gly | f | Probably damaging / Damaging | Clarke *et al*. 2008 (Fam 1) |
| Fam 29* | F | Cap | exon 8 | all | AD *de novo* | c.734G>T | (p.Arg244Ile) p.Arg245Ile | f | Probably damaging / Damaging | Present study |
| Fam 30* | F | CM | exon 8 | all | AD *de novo* | c.758C>A | (p.Thr252Lys) | g | Possibly | Present study |

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| | | | | | | | p.Thr253Lys | | damaging / Tolerated | |
| Fam 31 | M | NM severe | exon 1 | all | AR homozygous | c.94C>T | (p.Gln31Stop) p.Gln32Stop | c | - | Tan et al. 1999 |
| Fam 32 | F | NM | intron 9 | muscle | AR | c.915A>C | (p.Stop284Ser) p.Stop285Ser | d | - | Wattana-sirichaigoon *et al*. 2002 |
| Fam 33 | F | CFTD | exon 9 | muscle | AR | c.857A>C | (p.Stop284Ser) p.Stop285Ser | d | - | Lawlor et al. 2010 |
| Fam 34 and 35 | F, M | NM severe | exon 9b | muscle | AR | c.913delA | (p.Stop284Asn) p.Stop285Asn extStop74 | d | - | Lehtokari *et al*. 2008 |

PolyPhen-2 and FATHMM predictions indicate the possible pathogenicity of the missense mutations. Mutation nomenclature used in some publications is shown in brackets. The possible pathogenicity of the mutations was assessed with PolyPhen-2, version 2.2.2, HumVar option, and FATHMM version 2.3 with the prediction algorithm weighted for human mutations and disease ontology as the option for phenotypic associations. Nucleotide numbering according to the coding sequence of the *TPM3* cDNA reference sequence NM_152263.3. The first 2 ATG codons in the primary cDNA were both included according to the Human Genome Variation Society recommendations. Amino acid coordinates are provided relative to NP_689476.2, the ATG translation initiation codon is codon 1. Abbreviations: NM, nemaline myopathy; Cap, cap myopathy; CFTD, congenital fibre type disproportion; CM, undefined congenital myopathy; G, gender; AD, autosomal dominant; AR, autosomal recessive; L, location in heptad repeat; spl, splice site mutation; * new mutation; # gain-of-function mutation resulting in hypercontractile phenotypes.

**Supp. Table S5. *TPM2* mutations of congenital myopathy patients lacking clinical details in 12 families**

| Family | Disease | Location | Mutation(s) in cDNA | Altered protein site and predicted effect | L | PolyPhen-2 / FATHMM prediction | Reference |
|---|---|---|---|---|---|---|---|
| Fam 1* | - | exon 1a | c.5A>T | p.Asp2Val | b | Benign / Damaging | Present study |
| Fam 2* | - | exon2 | c.124G>A | p.Glu41Lys | f | Benign / Tolerated | Present study |
| Fam 3* | DA | exon 3 | c.240+4_+7del AGTG | spl | - | - | Present study |
| Fam 4* | DA | exon 3 | c.278A>G | p.Gln93Arg | b | Possibly damaging / Damaging | Present study |
| Fam 5* | - | exon 4 | c.398G>C | p.Arg133Pro | g | Probably damaging /Damaging | Present study |
| Fam 6* | - | exon 4 | c.463G>A | p.Ala155Thr | a | Probably damaging /Damaging | Present study |
| Fam 7* | - | exon 4 | c.463G>A | p.Ala155Thr | a | Probably damaging / Damaging | Present study |
| Fam 8* | - | exon 7 | c.654_656del | p.Glu218del | a | - | Present study |
| Fam 9* | - | exon 7 | c.654_656del | p.Glu218del | a | - | Present study |
| Fam 10* | Severe DA | exon 9a | c.773-3C>A | - | - | - | Present study |
| Fam 11* | - | exon 9a | c.782A>G | p.Tyr261Cys | b | Possibly damaging / Damaging | Present study |
| Fam 12* | DA | exon 9a | c.782A>G | p.Tyr261Cys | b | Possibly damaging / Damaging | Present study |

The possible pathogenicity of the mutations was assessed with PolyPhen-2, version 2.2.2, HumVar option, and FATHMM version 2.3 with the prediction algorithm weighted for human mutations and disease ontology as the option for phenotypic associations. Nucleotide numbering according to the *TPM2* cDNA sequence with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence NM_003289.3. Amino acid coordinates are provided relative to NP_003280.2, the ATG translation initiation codon is codon 1. Abbreviations: DA, distal arthrogryposis; L, location in heptad repeat; spl, splice site mutation; * new mutation

**Supp. Table S6. *TPM3* mutations of congenital myopathy patients lacking clinical details**

| **Family *Tpm3* mutations** | **Disease** | **Location** | **Mutation(s) in cDNA RefSeq NM152263** | **Altered protein site and predicted effect** | **L** | **Reference** |
|---|---|---|---|---|---|---|
| Fam 1 | CM | exon 5 | c.502C>T | p.Arg168Cys | f | Present study |
| Fam 2 | CM | exon 5 | c.502C>T | p.Arg168Cys | f | Present study |
| Fam 3 | CFTD | exon 5 | c.502C>T | p.Arg168Cys | f | Present study |
| Fam 4 | - | exon 5 | c.503G>A | p.Arg168His | f | Present study |
| Fam 5 | - | exon 5 | c.503G>A | p.Arg168His | f | Present study |

Nucleotide numbering according to the coding sequence of the *TPM3* cDNA reference sequence NM_152263.3. The first 2 ATG codons in the primary cDNA were both included according to the Human Genome Variation Society recommendations. Amino acid coordinates are provided relative to NP_689476.2, the ATG translation initiation codon is codon 1. Abbreviations: CFTD, congenital fibre type disproportion; CM, undefined congenital myopathy; L, location in heptad repeat

**Supp. Table S7. Phosphorylation sites in β-tropomyosin (Tm2)**

| **Human** | | **Mouse** | | **Present study** | | | |
|---|---|---|---|---|---|---|---|
| *Site* | *Reference* | *Site* | *Reference* | *Known site* | *IS* | *Novel site* | *IS* |
| T53-P | www.phosphosite.org | S87-P | Huttlin, E.L. 2010; Hsu, P.P. 2011 | T53-P | 46 | T79-P | 69 |
| S63-P | www.phosphosite.org | Y162-P | www.phosphosite.org | T252-P | 59 | T108-P | 60 |
| S87-P | Brill, L.M. 2009 | T252-P | Dai, J. 2007 | T282-P | 74 | S158-P | 58 |
| Y162-P | Rikova, K. 2007 | Y261-P | www.phosphosite.org | S283-P | 48 | S206-P | 56 |
| Y261-P | Iliuk, A.B. 2010 | T282-P | Dai, J. 2007; Huttlin, E.L. 2010 | | | | |
| | | S283-P | Dai, J. 2007; Zanivan, S. 2008; Huttlin, E.L. 2010 | | | | |

The novel sites were found in both wild type Tm2 and five mutants, Glu41Lys, Lys49del, Glu117Lys, Glu139del and Gln147Pro. IS represents the ion score in the Mascot search and the threshold value 40 was chosen.