

**CUGC for Nemaline myopathy**

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## **Clinical utility gene card for: Nemaline myopathy**

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# 1. Disease characteristics

## 1.1 Name of the Disease (Synonyms):

Nemaline myopathy (NEM1 – NEM9)

Includes nemaline myopathy with excess thin filaments/actin aggregates; nemaline myopathy with cores; nemaline myopathy with intranuclear rods; and Amish nemaline myopathy.

## 1.2 OMIM# of the Disease:

*NEM1* - 609284; *NEM2* - 256030; *NEM3* - 161800; *NEM4* - 609285; *NEM5* - 605355; *NEM6* - 609273; *NEM7* - 610687; *NEM8* - 615348; *NEM9* - 615731

## 1.3 Name of the Analysed Genes or DNA/Chromosome Segments:

*Slow muscle  $\alpha$ -tropomyosin (TPM3)* - *NEM1*.

*Nebulin (NEB)* - *NEM2*.

*Skeletal muscle  $\alpha$ -actin (ACTA1)* - *NEM3*.

*$\beta$ -tropomyosin (TPM2)* - *NEM4*.

*Slow muscle troponin-T (TNNT1)* - *NEM5*.

*Kelch-repeat and BTB (POZ) Domain containing 13 (KBTBD13)* - *NEM6*.

*Skeletal muscle cofilin (CFL2)* - *NEM7*.

*KELCH-like 40 (KLHL40)* - *NEM8*.

*KELCH-like 41 (KLHL41)* - *NEM9*.

*Leiomodin 3 (LMOD3)*.

## 1.4 OMIM# of the Gene(s):

*TPM3* = \*191030; *NEB* = \*161650; *ACTA1* = \*102610; *TPM2* = \*190990; *TNNT1* = \*191041; *KBTBD13* = \*613727; *CFL2* = \*601443; *KLHL40* = \*615340; *KLHL41* = \*607701; *LMOD3* = \*616112.

## 1.5 Mutational Spectrum:

**TPM3:** Mainly dominant, missense variants,<sup>1,2</sup> however some recessive variants have been described.<sup>3,4</sup> A 1bp recessive deletion occurs as a founder variant in the Turkish population.<sup>5</sup>

**NEB:** All of the over 140 variants identified to date are recessive and the patients are usually compound heterozygous. The majority of the variants are either frameshift or nonsense variants, but also missense variants, and point variants and deletions affecting splice sites are known<sup>6,7</sup>. An in-frame deletion of exon 55 is present in the Ashkenazi Jewish population at a carrier frequency of approximately 1 in 108.<sup>8</sup> Some patients present with a distal myopathy, with their skeletal muscle biopsies containing nemaline bodies, both nemaline bodies and cores, or no nemaline bodies.<sup>9,10</sup> Rare cases of core-rod myopathy with generalised muscle weakness may also be caused by *NEB* variants.<sup>11</sup>

**ACTA1:** Over 200 different variants identified, with the majority causing nemaline myopathy, or nemaline myopathy with other features (eg cores, actin aggregates, intranuclear rods, zebra bodies).<sup>12</sup> Of these, most variants are dominant, missense, and have arisen *de novo*.<sup>13</sup> About 10% are recessive variants. Most recessive variants are genetic or functional null variants<sup>13</sup> but recently recessive *ACTA1* disease with retention of skeletal muscle actin expression was described in a family presenting with a rigid spine syndrome.<sup>14</sup> Dominant inheritance is less common, and only seen in families with a milder phenotype.<sup>13</sup> Somatic mosaicism is possible with dominant variants.<sup>15</sup> Some variants can cause hypercontractile skeletal muscle rather than weakness.<sup>16</sup>

**TPM2:** Two heterozygous, dominant missense variants causing nemaline myopathy are known.<sup>17</sup> Also a homozygous null variant in a patient with nemaline and Escobar syndrome,<sup>18</sup> and a dominant heterozygous variant in a mother with nemaline myopathy and her daughter with cap myopathy<sup>19</sup> have been identified. A K7del variant was identified in a family with nemaline bodies and minicores (presenting as a distal myopathy), and also in four unrelated families with distal arthrogyrosis type 7 with nemaline bodies.<sup>20</sup> The K7del variant also causes progressive skeletal muscle contractures, most probably due to hypercontractility caused by the mutant protein, in nemaline myopathy and/or core rod myopathy patients.<sup>21</sup>

**TNNT1:** A recessive nonsense founder variant is present in the Old Order Amish population. This produces a characteristic progressive nemaline myopathy with tremors and contractures.<sup>22</sup> *TNNT1* variants have now been identified outside the Amish population.<sup>23</sup>

**KBTBD13:** Three dominant missense variants have been identified.<sup>24</sup> There was phenotypic variability, with some variant carriers exhibiting only very mild proximal leg weakness on targeted examination.

**CFL2:** Homozygous missense variants have been identified in two families,<sup>25,26</sup> and a homozygous 4 bp deletion identified in another family.<sup>27</sup> The severity of the disease is greater in the family with the homozygous null variant.

**KLHL40:** Homozygous or compound heterozygous variants (mainly missense, but also frameshift, splice site and nonsense variants) cause severe autosomal recessive nemaline myopathy with prenatal onset of symptoms, including fetal akinesia or hypokinesia and contractures.<sup>28</sup> At birth, the children may have fractures, respiratory failure, and severe swallowing difficulties.<sup>28</sup>

**KLHL41:** Recessive homozygous or compound heterozygous variants have been reported, with frameshift variants leading to a severe phenotype with neonatal death, and missense variants compatible with survival into late childhood or early adulthood.<sup>29</sup>

**LMOD3:** Recessive homozygous and compound heterozygous variants cause severe, usually lethal nemaline myopathy.<sup>30</sup>

Regularly updated variant databases exist for *ACTA1*, *CFL2*, *KBTBD13*, *KLHL40*, *NEB*, *TNNT1*, *TPM2* and *TPM3* at the Leiden Muscular Dystrophy pages© (<http://www.dmd.nl>).

## 1.6 Analytical Methods:

The main analytical method has been bi-directional Sanger sequencing of the entire coding region of the individual genes. If the family structure is amenable, linkage analysis for *NEB* may be useful to pre-screen, due to the large size of the gene. Next generation sequencing now allows for simultaneous analysis of all genes in a patient through whole exome sequencing. Alternatively sub-exomic sequencing using a panel of selected genes can include all known nemaline myopathy genes.<sup>31</sup> It should be noted however that limitations of current high throughput sequencing technologies prevent complete screening of all exons in all genes (e.g. regions of genes that have high GC content or are repetitive).

## 1.7 Analytical Validation

Variants should be confirmed by resequencing using a fresh dilution of genomic DNA. Putative variants identified through next generation sequencing methods should be confirmed by Sanger sequencing. Special care should be taken in interpreting missense variants in the nebulin gene as affecting function.<sup>32</sup>

## 1.8 Estimated Frequency of the Disease

(Incidence at birth ("birth prevalence") or population prevalence):

For the most part, the frequency of the disease is unknown. In Finland, the birth incidence has been estimated to be 0.02 per 1000 live births.<sup>33</sup> *De novo* dominant variants in *ACTA1* and recessive variants in *NEB* are the most common causes of NEM.<sup>34,35</sup>

## 1.9 If applicable, prevalence in the ethnic group of investigated person:

Recessive founder variants are known to exist in particular genes in specific populations: *TNNT1* in the Amish<sup>22</sup>; *NEB* in the Ashkenazi Jewish<sup>8</sup> and in the Finnish population.<sup>10</sup> *ACTA1* in the Pakistani community in England, and in French and Spanish Roma;<sup>13</sup> *TPM3* in the Turkish population;<sup>5</sup> and *KLHL40* in Japanese persons.<sup>28</sup> These specific cases aside, no clear differences in prevalence rates are known between different ethnic groups.

## 1.10 Diagnostic Setting:

	Yes.	No.
A. (Differential) diagnostics	<input checked="" type="checkbox"/>	<input type="checkbox"/>
B. Predictive Testing	<input checked="" type="checkbox"/>	<input type="checkbox"/>
C. Risk assessment in Relatives	<input checked="" type="checkbox"/>	<input type="checkbox"/>

D. Prenatal



Comment: Requests for predictive testing are not common because of the early onset of the disease, but may be offered in families with childhood or late-onset forms of the disease.

## 2. Test characteristics

		genotype or disease	
		present	absent
test	pos.	A	B
	neg.	C	D

A: true positives      C: false negatives  
B: false positives    D: true negatives

sensitivity:  $A/(A+C)$   
specificity:  $D/(D+B)$   
pos. predict. value:  $A/(A+B)$   
neg. predict. value:  $D/(C+D)$

### 2.1 Analytical Sensitivity

(proportion of positive tests if the genotype is present)

100%

### 2.2 Analytical Specificity

(proportion of negative tests if the genotype is not present)

100%

### 2.3 Clinical Sensitivity

(proportion of positive tests if the disease is present)

The clinical sensitivity is dependent on factors such as age, inheritance pattern and additional clinical features. Because of the genetic heterogeneity, and particularly the difficulty of screening *NEB*, full screening of all known nemaline myopathy genes has historically not been possible. However next generation technologies are now getting closer to such screening. If full screening were to be undertaken, it may be estimated that approximately 75% of patients would have a variant/s identified.<sup>6,13</sup>

### 2.4 Clinical Specificity

(proportion of negative tests if the disease is not present)

The clinical specificity can be dependent on variable factors such as age or family history. In such cases a general statement should be given, even if a quantification can only be made case by case.

Probably 100%.

### 2.5 Positive clinical predictive value

(life time risk to develop the disease if the test is positive).

Near 100%. Potential incomplete penetrance has been suggested for certain *ACTA1* variants<sup>36</sup> and a very mild phenotype can be associated with some *KBTBD13* variants.<sup>24</sup>

### 2.6 Negative clinical predictive value

(Probability not to develop the disease if the test is negative).

Assume an increased risk based on family history for a non-affected person. Allelic and locus heterogeneity may need to be considered.

Index case in that family had been tested:

Approximately 100%.

Index case in that family had not been tested:

No predictive tests are usually performed in such cases.

### 3. Clinical Utility

#### 3.1 (Differential) diagnostics: The tested person is clinically affected

(To be answered if in 1.10 "A" was marked)

##### 3.1.1 Can a diagnosis be made other than through a genetic test?

No.  (continue with 3.1.4)

Yes,

clinically.

imaging.

especially in uncertain/ambiguous cases.

endoscopy.

biochemistry.

electrophysiology.

other (please describe):  Histopathology, including electron microscopy of skeletal muscle.

*Besides 'pure' nemaline myopathy, variants in the known NEM genes have also been described in association with other congenital myopathies, which clinically can appear similar to nemaline myopathy. Pathologically, some patients have features in addition to nemaline bodies (eg cores<sup>37</sup>). Patients with variants in the NEM genes may also have a myopathy without nemaline bodies, and may be diagnosed as having actin myopathy (ACTA1<sup>15</sup>), intranuclear rod myopathy (ACTA1 eg<sup>38</sup>), congenital fibre type disproportion (OMIM #255310; ACTA1;<sup>39</sup> TPM3<sup>40,41</sup>), cap disease (ACTA1;<sup>42</sup> TPM2;<sup>43,44</sup> TPM3<sup>45,46</sup>) and distal myopathy (NEB<sup>47,48</sup>). Variants in TPM2 and other nemaline myopathy genes can also cause distal arthrogyrosis.<sup>49</sup>*

*Variants in other genes may also be associated with these additional histological features, e.g. nemaline myopathy with cores with variants in RYR1,<sup>50</sup> and congenital fibre type disproportion with RYR1<sup>51</sup> or SEPN1<sup>52</sup> variants. Variants in TPM2 or TPM3 can cause congenital myopathies with neuromuscular transmission defects that can benefit from treatment with anticholinesterase therapy.<sup>53,54</sup>*

*Nemaline bodies can also be identified as secondary features, such as in mitochondrial disorders<sup>55</sup> and HIV.<sup>56</sup>*

##### 3.1.2 Describe the burden of alternative diagnostic methods to the patient

Nemaline myopathy is both a clinical and, significantly, a histopathological/electron microscopic diagnosis. Therefore, a thorough assessment including a detailed evaluation of clinical and pathological features should be performed along with genetic testing. As such, histopathology and electron microscopy are not diagnostic alternatives, rather prerequisites to genetic testing. Nevertheless, muscle biopsy is an invasive procedure, and appropriate histological and electron microscopic examination requires proximity to a specialised laboratory set-up.

##### 3.1.3 How is the cost effectiveness of alternative diagnostic methods to be judged?

Not applicable.

### 3.1.4 Will disease management be influenced by the result of a genetic test?

No.

Yes.

Therapy (please describe)

Presently, the treatment of patients is based on symptom management, which would occur regardless of the genetic diagnosis. However an accurate genetic diagnosis will undoubtedly be extremely important for any causal therapies once they are developed.

Prognosis (please describe)

Varied, as nemaline myopathy can be severe (fetal or congenital presentation) through to mild,<sup>57</sup> even with variants in the same gene. For example, most patients with *ACTA1* variants affecting function die within their first year of life, whilst some patients have a milder phenotype compatible with survival into adulthood.<sup>13</sup> However, even severely affected patients who survive the first year of life may improve functionally in subsequent years. Patients with the recessive *TNNT1* variant die within 2 years of birth. The “typical” subtype of nemaline myopathy is considered non-progressive.

Management (please describe)

Supportive - mechanical ventilation; night-time ventilation; naso-gastric feeding; mobility aids; physiotherapy, occupational and speech therapy; surgery for scoliosis where needed. Cardiomyopathy is not normally present, however some instances have been reported with *ACTA1* variants. E.g.<sup>58</sup> Genetic testing is required for verifying the diagnosis and determining the mode of inheritance, which serves as a basis for genetic counselling.

### **3.2 Predictive Setting: The tested person is clinically unaffected but carries an increased risk based on family history**

(To be answered if in 1.10 "B" was marked)

Predictive testing is usually only applicable for the milder versions of nemaline myopathy, as most often the disease presents before, at, or shortly after birth.

#### **3.2.1 Will the result of a genetic test influence lifestyle and prevention?**

If the test result is **positive** (please describe)

The positive test result may influence lifestyle choices for later onset disease, for example deciding which career to pursue (eg an office job compared to something physical), whether to travel at a young age when mobility is not affected compared to later in life, and whether to have a house on one level as opposed to over multiple stories (eg the latter would involve climbing lots of stairs which might become problematic). Respiratory precautions and self-monitoring for sleep-related breathing problems may be advised. A positive test will greatly influence prevention, through the availability of prenatal or preimplantation diagnosis for the family.

If the test result is **negative** (please describe)

A negative test result does not rule out the diagnosis of nemaline myopathy due to the possibility of currently unidentified causative genes or pathogenic variants not being identified in, for example, regions of NEB that are difficult to sequence and/or map in next generation sequencing.<sup>31</sup> (Kiiski et al., submitted). In this situation, prevention through genetic counselling is less informed. In terms of predictive testing, a negative result may influence lifestyle choices as indicated above, such as choice of occupation.

#### **3.2.2 Which options in view of lifestyle and prevention does a person at-risk have if no genetic test has been done (please describe)?**

Not applicable.

### **3.3 Genetic risk assessment in family members of a diseased person**

(To be answered if in 1.10 "C" was marked)

#### **3.3.1 Does the result of a genetic test resolve the genetic situation in that family?**

Yes, if a variant/s is identified.

#### **3.3.2 Can a genetic test in the index patient save genetic or other tests in family members?**

Genetic testing would still most likely be performed for other family members, however other tests such as skeletal muscle biopsy might be prevented.

#### **3.3.3 Does a positive genetic test result in the index patient enable a predictive test in a family member?**

Yes, but due to very early disease onset in most cases, it is infrequently encountered.

### **3.4 Prenatal diagnosis**

(To be answered if in 1.10 "D" was marked)

#### **3.4.1 Does a positive genetic test result in the index patient enable a prenatal diagnosis?**

Yes. In cases where dominant de novo variants have been identified in an affected child, genetic counselling may be difficult, as the recurrence risk is between 0 and 25% due to the possibility of gonadal mosaicism. However, prenatal genetic testing of an at-risk pregnancy is accurate.



#### **4. If applicable, further consequences of testing**

Please assume that the result of a genetic test has no immediate medical consequences. Is there any evidence that a genetic test is nevertheless useful for the patient or his/her relatives? (Please describe)

Yes, particularly if a variant/s are identified. An accurate genetic diagnosis often ends a lengthy diagnostic odyssey for the patient and their family, removing the psychological affects of an absent disease cause, and can sometimes influence possible prognosis. An accurate genetic diagnosis can crucially indicate mode of inheritance and underpins genetic counselling, including options such as prenatal and pre-implantation testing.

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#### **Conflict of Interest**

The authors declare they have no conflicts of interest.

## References

1. Laing NG, Wilton SD, Akkari PA *et al*: A mutation in the alpha tropomyosin gene TPM3 associated with autosomal dominant nemaline myopathy. *Nat Genet* 1995; **9**: 75-79.
2. Kiphuth IC, Krause S, Huttner HB, Dekomien G, Struffert T, Schroder R: Autosomal dominant nemaline myopathy caused by a novel alpha-tropomyosin 3 mutation. *J Neurol* 2010; **257**: 658-660.
3. Tan P, Briner J, Boltshauser E *et al*: Homozygosity for a nonsense mutation in the alpha-tropomyosin slow gene TPM3 in a patient with severe infantile nemaline myopathy. *Neuromuscul Disord* 1999; **9**: 573-579.
4. Wattanasirichaigoon D, Swoboda KJ, Takada F *et al*: Mutations of the slow muscle alpha-tropomyosin gene, TPM3, are a rare cause of nemaline myopathy. *Neurology* 2002; **59**: 613-617.
5. Lehtokari VL, Pelin K, Donner K *et al*: Identification of a founder mutation in TPM3 in nemaline myopathy patients of Turkish origin. *Eur J Hum Genet* 2008; **16**: 1055-1061.
6. Pelin K, Hilpela P, Donner K *et al*: Mutations in the nebulin gene associated with autosomal recessive nemaline myopathy. *Proc Natl Acad Sci U S A* 1999; **96**: 2305-2310.
7. Lehtokari VL, Pelin K, Sandbacka M *et al*: Identification of 45 novel mutations in the nebulin gene associated with autosomal recessive nemaline myopathy. *Hum Mutat* 2006; **27**: 946-956.
8. Anderson SL, Ekstein J, Donnelly MC *et al*: Nemaline myopathy in the Ashkenazi Jewish population is caused by a deletion in the nebulin gene. *Hum Genet* 2004; **115**: 185-190.
9. Scoto M, Cullup T, Cirak S *et al*: Nebulin (NEB) mutations in a childhood onset distal myopathy with rods and cores uncovered by next generation sequencing. *European journal of human genetics : EJHG* 2013; **21**: 1249-1252.
10. Lehtokari VL, Kiiski K, Sandaradura SA *et al*: Mutation update: the spectra of nebulin variants and associated myopathies. *Hum Mutat* 2014; **35**: 1418-1426.
11. Romero NB, Lehtokari VL, Quijano-Roy S *et al*: Core-rod myopathy caused by mutations in the nebulin gene. *Neurology* 2009; **73**: 1159-1161.
12. Nowak KJ, Ravenscroft G, Laing NG: Skeletal muscle alpha-actin diseases (actinopathies): pathology and mechanisms. *Acta Neuropathologica* 2013; **125**: 12-29.
13. Laing NG, Dye DE, Wallgren-Pettersson C *et al*: Mutations and polymorphisms of the skeletal muscle alpha-actin gene (ACTA1). *Hum Mutat* 2009; **30**: 1267-1277.
14. O'Grady GL, Best HA, Oates EC *et al*: Recessive ACTA1 variant causes congenital muscular dystrophy with rigid spine. *Eur J Hum Genet* 2014; **In press**.
15. Nowak KJ, Wattanasirichaigoon D, Goebel HH *et al*: Mutations in the skeletal muscle alpha-actin gene in patients with actin myopathy and nemaline myopathy. *Nat Genet* 1999; **23**: 208-212.
16. Jain RK, Jayawant S, Squier W *et al*: Nemaline myopathy with stiffness and hypertonia associated with an ACTA1 mutation. *Neurology* 2012; **78**: 1100-1103.
17. Donner K, Ollikainen M, Ridanpaa M *et al*: Mutations in the beta-tropomyosin (TPM2) gene - a rare cause of nemaline myopathy. *Neuromuscul Disord* 2002; **12**: 151-158.

18. Monnier N, Lunardi J, Marty I *et al*: Absence of beta-tropomyosin is a new cause of Escobar syndrome associated with nemaline myopathy. *Neuromuscul Disord* 2009; **19**: 118-123.
19. Tajsharghi H, Ohlsson M, Lindberg C, Oldfors A: Congenital myopathy with nemaline rods and cap structures caused by a mutation in the beta-tropomyosin gene (*TPM2*). *Arch Neurol* 2007; **64**: 1334-1338.
20. Davidson AE, Siddiqui FM, Lopez MA *et al*: Novel deletion of lysine 7 expands the clinical, histopathological and genetic spectrum of *TPM2*-related myopathies. *Brain* 2013; **136**: 508-521.
21. Mokbel N, Ilkovski B, Kreissl M *et al*: K7del is a common *TPM2* gene mutation associated with nemaline myopathy and raised myofibre calcium sensitivity. *Brain* 2013; **136**: 494-507.
22. Johnston JJ, Kelley RI, Crawford TO *et al*: A novel nemaline myopathy in the Amish caused by a mutation in troponin T1. *Am J Hum Genet* 2000; **67**: 814-821.
23. van der Pol WL, Leijenaar JF, Spliet WG *et al*: Nemaline myopathy caused by *TNNT1* mutations in a Dutch pedigree. *Mol Genet Genomic Med* 2014; **2**: 134-137.
24. Sambuughin N, Yau KS, Olive M *et al*: Dominant mutations in *KBTBD13*, a member of the BTB/Kelch family, cause nemaline myopathy with cores. *Am J Hum Genet* **87**: 842-847.
25. Agrawal PB, Greenleaf RS, Tomczak KK *et al*: Nemaline myopathy with minicores caused by mutation of the *CFL2* gene encoding the skeletal muscle actin-binding protein, cofilin-2. *Am J Hum Genet* 2007; **80**: 162-167.
26. Ockeloen CW, Gilhuis HJ, Pfundt R *et al*: Congenital myopathy caused by a novel missense mutation in the *CFL2* gene. *Neuromuscul Disord* 2012; **22**: 632-639.
27. Ong RW, Alsaman A, Selcen D *et al*: Novel cofilin-2 (*CFL2*) four base pair deletion causing nemaline myopathy. *J Neurol Neurosurg, Psychiatry* 2014; **85**: 1058-1060
28. Ravenscroft G, Miyatake S, Lehtokari VL *et al*: Mutations in *KLHL40* are a frequent cause of severe autosomal-recessive nemaline myopathy. *Am J Hum Genet* 2013; **93**: 6-18.
29. Gupta VA, Ravenscroft G, Shaheen R *et al*: Identification of *KLHL41* mutations implicates BTB-Kelch-mediated ubiquitination as an alternate pathway to myofibrillar disruption in nemaline myopathy. *Am J Hum Genet* 2013; **93**: 1108-1117.
30. Yuen M, Sandaradura SA, Dowling JJ *et al*: Leiomodlin-3 dysfunction results in thin filament disorganization and nemaline myopathy. *J Clin Invest* 2014; **124**: 4693-4708.
31. Kiiski K, Laari L, Lehtokari VL *et al*: Targeted array comparative genomic hybridization-a new diagnostic tool for the detection of large copy number variations in nemaline myopathy-causing genes. *Neuromuscul Disord* 2013; **23**: 56-65.
32. Lehtokari VL, Kiiski K, Sandaradura SA *et al*: Mutation update: the spectra of nebulin variants and associated myopathies. *Hum Mutat* 2014; **35**:1418-1426.
33. Wallgren-Pettersson C: Congenital nemaline myopathy: a longitudinal study. University of Helsinki, Helsinki, 1990.
34. Wallgren-Pettersson C, Pelin K, Hilpela P *et al*: Clinical and genetic heterogeneity in autosomal recessive nemaline myopathy. *Neuromuscul Disord* 1999; **9**: 564-572.
35. Wallgren-Pettersson C, Laing NG: Report of the 83rd ENMC International Workshop: 4th Workshop on Nemaline Myopathy, 22-24 September 2000, Naarden, The Netherlands. *Neuromuscul Disord* 2001; **11**: 589-595.

36. Agrawal PB, Strickland CD, Midgett C *et al*: Heterogeneity of nemaline myopathy cases with skeletal muscle alpha-actin gene mutations. *Ann Neurol* 2004; **56**: 86-96.
37. Jungbluth H, Sewry CA, Brown SC *et al*: Mild phenotype of nemaline myopathy with sleep hypoventilation due to a mutation in the skeletal muscle alpha-actin (*ACTA1*) gene. *Neuromuscul Disord* 2001; **11**: 35-40.
38. Hutchinson DO, Charlton A, Laing NG, Ilkovski B, North KN: Autosomal dominant nemaline myopathy with intranuclear rods due to mutation of the skeletal muscle *ACTA1* gene: clinical and pathological variability within a kindred. *Neuromuscul Disord* 2006; **16**: 113-121.
39. Laing NG, Clarke NF, Dye DE *et al*: Actin mutations are one cause of congenital fibre type disproportion. *Ann Neurol* 2004; **56**: 689-694.
40. Clarke NF, Kolski H, Dye DE *et al*: Mutations in *TPM3* are a common cause of congenital fiber type disproportion. *Ann Neurol* 2008; **63**: 329-337.
41. Lawlor MW, Dechene ET, Roumm E, Geggel AS, Moghadaszadeh B, Beggs AH: Mutations of tropomyosin 3 (*TPM3*) are common and associated with type 1 myofiber hypotrophy in congenital fiber type disproportion. *Hum Mutat* 2010; **31**: 176-183.
42. Hung RM, Yoon G, Hawkins CE, Halliday W, Biggar D, Vajsar J: Cap myopathy caused by a mutation of the skeletal alpha-actin gene *ACTA1*. *Neuromuscul Disord* 2010; **20**: 238-240.
43. Lehtokari VL, Ceuterick-de Groote C, de Jonghe P *et al*: Cap disease caused by heterozygous deletion of the beta-tropomyosin gene *TPM2*. *Neuromuscul Disord* 2007; **17**: 433-442.
44. Clarke NF, Domazetovska A, Waddell L, Kornberg A, McLean C, North KN: Cap disease due to mutation of the beta-tropomyosin gene (*TPM2*). *Neuromuscul Disord* 2009; **19**: 348-351.
45. Ohlsson M, Fidzianska A, Tajsharghi H, Oldfors A: *TPM3* mutation in one of the original cases of cap disease. *Neurology* 2009; **72**: 1961-1963.
46. De Paula AM, Franques J, Fernandez C *et al*: A *TPM3* mutation causing cap myopathy. *Neuromuscul Disord* 2009; **19**: 685-688.
47. Wallgren-Pettersson C, Lehtokari VL, Kalimo H *et al*: Distal myopathy caused by homozygous missense mutations in the nebulin gene. *Brain* 2007; **130**: 1465-1476.
48. Lehtokari VL, Pelin K, Herczegfalvi A *et al*: Nemaline myopathy caused by mutations in the nebulin gene may present as a distal myopathy. *Neuromuscul Disord* 2011; **21**: 556-562.
49. Sung SS, Brassington AM, Grannatt K *et al*: Mutations in genes encoding fast-twitch contractile proteins cause distal arthrogyrosis syndromes. *Am J Hum Genet* 2003; **72**: 681-690.
50. Scacheri PC, Hoffman EP, Fratkin JD *et al*: A novel ryanodine receptor gene mutation causing both cores and rods in congenital myopathy. *Neurology* 2000; **55**: 1689-1696.
51. Clarke NF, Waddell LB, Cooper ST *et al*: Recessive mutations in *RYR1* are a common cause of congenital fiber type disproportion. *Hum Mutat* 2010; **31**: E1544-1550.
52. Clarke NF, Kidson W, Quijano-Roy S *et al*: *SEPN1*: associated with congenital fiber-type disproportion and insulin resistance. *Ann Neurol* 2006; **59**: 546-552.
53. Munot P, Lashley D, Jungbluth H *et al*: Congenital fibre type disproportion associated with mutations in the tropomyosin 3 (*TPM3*) gene mimicking congenital myasthenia. *Neuromuscul Disord* 2010; **20**: 796-800.

54. Rodriguez Cruz PM, Sewry C, Beeson D *et al*: Congenital myopathies with secondary neuromuscular transmission defects; A case report and review of the literature. *Neuromuscul Disord* 2014; **24**: 1103-1110.
55. Bethlem J, Arts WF, Dingemans KP: Common origin of rods, cores, miniature cores, and focal loss of cross-striations. *Arch Neurol* 1978; **35**: 555-566.
56. Feinberg DM, Spiro AJ, Weidenheim KM: Distinct light microscopic changes in human immunodeficiency virus-associated nemaline myopathy. *Neurology* 1998; **50**: 529-531.
57. Wallgren-Pettersson C, Laing NG: Report of the 70th ENMC International Workshop: nemaline myopathy, 11-13 June 1999, Naarden, The Netherlands. *Neuromuscul Disord* 2000; **10**: 299-306.
58. D'Amico A, Graziano C, Pacileo G *et al*: Fatal hypertrophic cardiomyopathy and nemaline myopathy associated with *ACTA1* K336E mutation. *Neuromuscul Disord* 2006; **16**: 548-552.

## Clinical utility gene card for: Nemaline myopathy

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### Name of the Disease (Synonyms):

Nemaline myopathy (NEM)

Includes nemaline myopathy with excess thin filaments/actin aggregates; nemaline myopathy with cores; nemaline myopathy with intranuclear rods; and Amish nemaline myopathy.

### OMIM# of the Disease:

*NEM1* - 609284; *NEM2* - 256030; *NEM3* - 161800; *NEM4* - 609285; *NEM5* - 605355; *NEM6* - 609273; *NEM7* - 610687; *NEM8* - 615348; *NEM9* - 615731

### Analysed Genes or DNA/Chromosome Segments:

*Slow muscle  $\alpha$ -tropomyosin (TPM3)* - *NEM1*.

*Nebulin (NEB)* - *NEM2*.

*Skeletal muscle  $\alpha$ -actin (ACTA1)* - *NEM3*.

*$\beta$ -tropomyosin (TPM2)* - *NEM4*.

*Slow muscle troponin-T (TNNT1)* - *NEM5*.

*Kelch-repeat and BTB (POZ) Domain containing 13 (KBTBD13)* - *NEM6*.

*Skeletal muscle cofilin (CFL2)* - *NEM7*.

*KELCH-like 40 (KLHL40)* - *NEM8*.

*KELCH-like 41 (KLHL41)* - *NEM9*.

*Leiomodin 3 (LMOD3)*.

### OMIM# of the Gene(s):

*TPM3* = \*191030; *NEB* = \*161650; *ACTA1* = \*102610; *TPM2* = \*190990; *TNNT1* = \*191041; *KBTBD13* = \*613727; *CFL2* = \*601443; *KLHL40* = \*615340; *KLHL41* = \*607701; *LMOD3* = \*616112.

Review of the analytical and clinical validity as well as of the clinical utility of DNA-based testing for variants in the *NEM1* – *NEM9* genes, and *LMOD3* in diagnostic, predictive and prenatal settings and for risk assessment in relatives.