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CLINICAL UTILITY GENE CARD

Clinical utility gene card for: Nemaline myopathy

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1. DISEASE CHARACTERISTICS

1.1 Name of the disease (synonyms)

Nemaline myopathy (NEM1 – NEM7)

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.

1.3 Name of the analysed genes or DNA/chromosome segments

Slow muscle α-tropomyosin (TPM3) – NEM1.
Nebulin (NEB) – NEM2.
Skeletal muscle α-actin (ACTA1) – NEM3.
β-tropomyosin (TPM2) – NEM4.
Slow muscle troponin-T (TNNT1) – NEM5.
Kelch-repeat and BTB (POZ) Domain containing 13 (KBTBD13) – NEM6.
Skeletal muscle cofilin (CFL2) – NEM7.

1.4 OMIM# of the gene(s)

TPM3 = *191030; *NEB* = *161650; *ACTA1* = *102610; *TPM2* = *190990; *TNNT1* = *191041; *KBTBD13* = *613727; *CFL2* = *601443.

1.5 Mutational spectrum

TPM3

Mainly dominant, missense mutations,[1,2] however some recessive mutations have been described.[3,4] A 1 bp recessive deletion occurs as a founder mutation in the Turkish population.[5]

NEB

All the over 140 mutations identified to date are recessive and the patients usually are compound heterozygous. The majority of the mutations are either frameshift or nonsense mutations, but also missense mutations, and point mutations and deletions affecting splice sites are known.[6,7] An in-frame deletion of exon 55 is present in the Ashkenazi Jewish population at a carrier frequency of approximately 1 in 108.[8]

ACTA1

Over 200 different mutations identified, with the majority causing nemaline myopathy, and nemaline myopathy with other features (for example, cores, actin aggregates, intranuclear rods).[9] Of these, most mutations are dominant, missense, and have arisen *de novo*.[9] About 10% are recessive mutations, and are genetic or functional null mutations.[9] Dominant inheritance is less common, and only seen in families with a milder phenotype.[9]

TPM2

Two heterozygous, dominant missense mutations causing nemaline myopathy are known.[10] Also a homozygous null mutation in a patient with nemaline and Escobar syndrome,[11] and a dominant heterozygous mutation in a mother with nemaline myopathy and her daughter with cap myopathy.[12]

TNNT1

A recessive nonsense founder mutation is present in the Old Order Amish population. This produces characteristic progressive nemaline myopathy with tremors and contractures.[13]

KBTBD13

Three dominant missense mutations have been identified; however, not all mutation carriers in the families exhibited skeletal muscle weakness.[14]

CFL2

A single homozygous missense mutation has been identified in one family.[15]

1.6 Analytical methods

The main analytical method is 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, owing to the large size of the gene. Next-generation sequencing will allow for simultaneous analysis of all genes in a patient.

1.7 Analytical validation

Mutations should be confirmed by resequencing using a fresh dilution of genomic DNA.

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 prevalence has been estimated to be 0.02 per 1000 live births.[16] Dominant mutations in *ACTA1* and recessive mutations in *NEB* are the most common causes of NEM.[17,18]

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

Recessive founder mutations are known to exist in particular genes in specific populations: *TNNT1* in the Amish;[13] *NEB* in the Ashkenazi Jewish;[8] *ACTA1* in the Pakistani community in England; and in French and Spanish Roma;[9] and *TPM3* in the Turkish population.[5] These specific cases aside, no clear differences exist in prevalence rates between different ethnicities.

1.10 Diagnostic setting

	Yes	No
A. (Differential) diagnostics	☒	☐
B. Predictive testing	☒	☐
C. Risk assessment in relatives	☒	☐
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		A: True positives B: False positives	C: False negative D: True negative
	Present	Absent		
Test				
Positive	A	B	Sensitivity:	A/(A + C)
			Specificity:	D/(D + B)
Negative	C	D	Positive predictive value:	A/(A + B)
			Negative predictive 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 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.

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 is rarely carried out. If full screening were to be undertaken, it may be estimated that approximately 75% of patients would have an identifiable mutation.[6,9]

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.[19]

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) diagnosis: 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	☒MRI imaging of muscles may be able to direct mutation testing, especially in uncertain/ambiguous cases.
	Endoscopy	☐
	Biochemistry	☐
	Electrophysiology	☐
	Other (please describe)	☒Histopathology and electron microscopy of skeletal muscle. Besides 'pure' nemaline myopathy, mutations 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[20]). Patients with mutations in the *NEM* genes may also have a myopathy without nemaline bodies, and may be diagnosed as having actin myopathy (*ACTA1*[21]), intranuclear rod myopathy (eg, *ACTA1*[22]), congenital fibre type disproportion (OMIM #255310; *ACTA1*;[23] *TPM3*[24,25]), cap disease (*ACTA1*;[26] *TPM2*;[27,28] *TPM3*[29,30]) and distal myopathy (*NEB*[31,32]). Mutations in *TPM2* and other nemaline myopathy genes can also cause distal arthrogryposis.[33] Mutations in other genes may also be associated with these additional phenotypes (eg, congenital fibre type disproportion and nemaline myopathy with cores can be caused by mutations in *RYR1*[34,35] and *SEPN1*.[36]

3.1.2 Describe the burden of alternative diagnostic methods to the patient

Nemaline myopathy requires 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 before 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 gene- or mutation-dependent therapies once they are developed.
	Prognosis (please describe)	Congenital myopathies are usually considered non-progressive; however, it is variable, as nemaline myopathy can be severe (congenital presentation) through to mild,[37] even with mutations in the same gene. For example, most patients with ACTA1 mutations die within their first year of life, but some patients have a milder phenotype compatible with survival into adulthood.[9] However, even severely affected patients who can get through the first year of life can do better subsequently. Patients with the recessive TNNTI mutation die within 2 years of birth.
	Management (please describe)	Supportive-mechanical ventilation; night-time ventilation; naso-gastric feeding; mobility aids; physio, occupational and speech therapy. Genetic testing 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):

A positive result may perhaps influence decisions in terms of lifestyle choices, for example, deciding which career to pursue (eg, an office job compared with a more physical occupation), whether to travel at a young age when mobility is not affected compared with later in life after the disease onsets, 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).

If the test result is negative (please describe):

A negative result may influence lifestyle choices in the opposite directions to those indicated above.

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 mutation/s is identified.

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

3.3.3 Does a positive genetic test result in the index patient enable a predictive test in a family member?
Yes, but owing 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* mutations have been identified in an affected child, genetic counselling may be difficult, as the recurrence risk is between 0 and 50%, and there is currently insufficient empirical data to indicate the risk. 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 mutation/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.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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